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18

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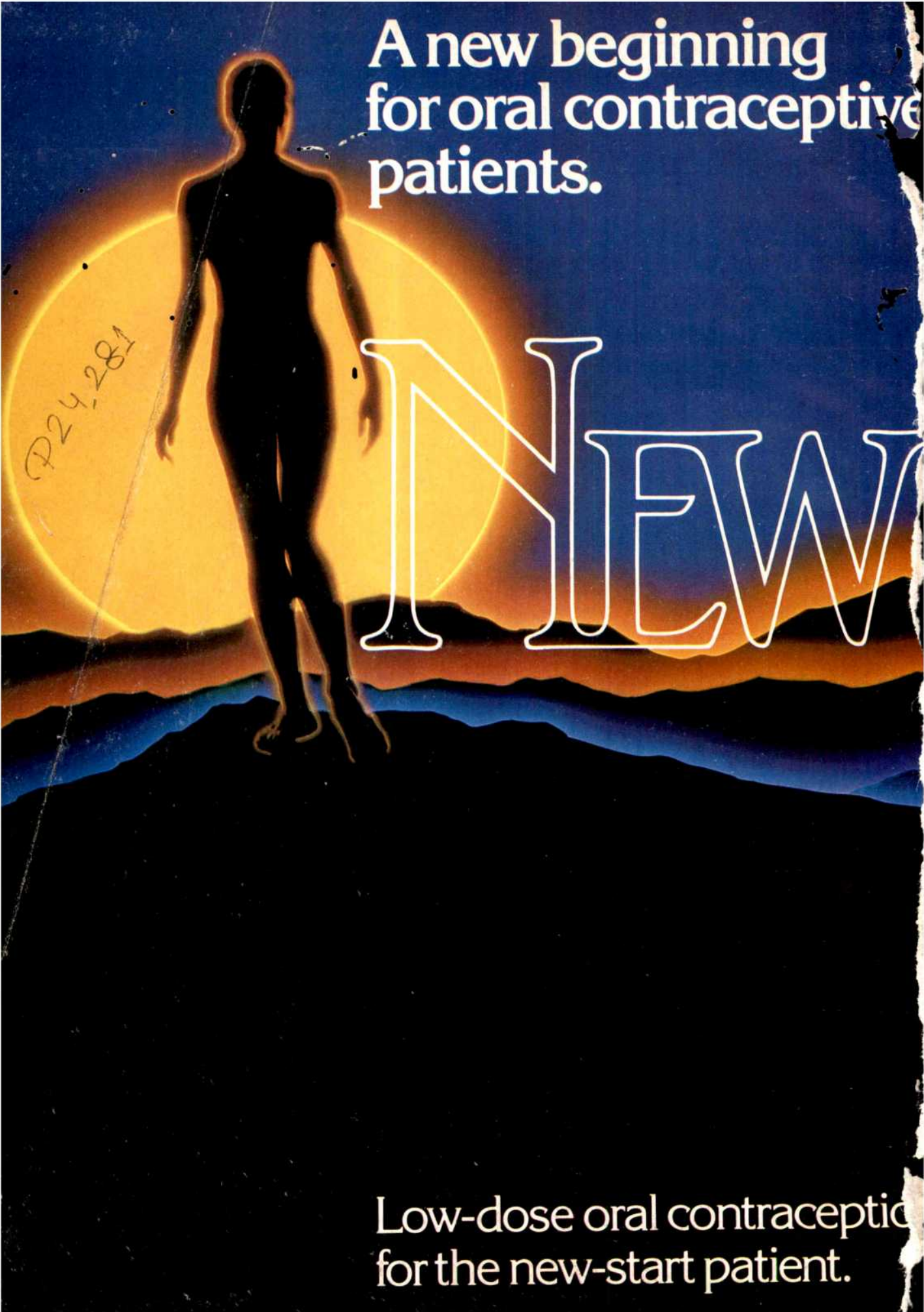
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\*Note: serious as well as minor side effects have been reported following the use of all oral contraceptives. These include thromboembolic disease. Please see summary of Prescribing Information on following page.

†The overall pregnancy rate was 0.17 per hundred woman-years, in clinical trials with 940 patients completing 14,366 cycles of use.



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**Norinyl® 1+35 28-Day** Tablets (21 norethindrone 1 mg, with ethinyl estradiol 0.035 mg. tablets followed by 7 inert tablets)

# **ORAL CONTRACEPTIVE (O.C.) AGENTS**

**Indications:** O.C.s are indicated for the prevention of pregnancy. **DOSE-RELATED RISK OF THROMBOEMBOLISM FROM O.C.s:** Studies have shown a positive association between the dose of estrogens in O.C.s and the risk of thromboembolism. For this reason, it is prudent and in keeping with good principles of therapeutics to minimize exposure to estrogen. The O.C. product prescribed for any given patient should be that product which contains the least amount of estrogen that is compatible with an acceptable pregnancy rate and patient acceptance. It is recommended that new acceptors of O.C.s should be started on preparations containing 0.05 mg or less of estrogen. **Contraindications:** 1. Known or suspected pregnancy (see Warning No. 5). 2. Thrombophlebitis or thromboembolic disorders. 3. A past history of deep vein thrombophlebitis or thromboembolic disorders. 4. Undiagnosed abnormal genital bleeding. 5. O.C.s should not be used by women who have or have had any of the following conditions: a. Cerebral vascular or coronary artery disease. b. Known or suspected carcinoma of the breast. c. Known or suspected estrogen dependent neoplasia. d. Benign or malignant liver tumor which developed during the use of oral contraceptives or other estrogen containing products.

**WARNINGS:** Cigarette smoking increases the risk of serious cardiovascular side effects from O.C. use. This risk increases with age and with heavy smoking (15 or more cigarettes per day) and is quite marked in women over 35 years of age. Women who use O.C.s should be strongly advised not to smoke.

The use of O.C.s is associated with increased risk of several serious conditions including thromboembolism, stroke, myocardial infarction, liver tumor, gall bladder disease, visual disturbances, fetal abnormalities, and hypertension. Practitioners prescribing O.C.s should be familiar with the following information relating to these risks.

1. **Thromboembolic Disorders and Other Vascular Problems:** An increased risk of thromboembolic and thrombotic disease associated with the use of O.C.s is well established. One British study demonstrated an increased relative risk for fatal venous thromboembolism; two British and three American studies demonstrated an increased relative risk for non-fatal venous thromboembolism. These studies estimate that users of O.C.s are 4 to 11 times more likely than nonusers to develop these diseases without evident cause. **CEREBROVASCULAR DISORDERS:** Two American studies demonstrated an increased relative risk for stroke, which had not been shown in prior British studies. In a collaborative American study of cerebrovascular disorders in women with and without predisposing causes, it was estimated that the relative risk of hemorrhagic stroke was 2.0 times greater in users than nonusers and the relative risk of thrombotic stroke was 4 to 5.5 times greater in users than in nonusers. A British long-term, follow-up study reported in 1976 a highly significant association between O.C. use and stroke. Another British long-term, follow-up study had suggested such an association in 1974, but the number of cases was too small to estimate the risk. **MYOCARDIAL INFARCTION:** An increased relative risk of myocardial infarction associated with the use of O.C.s has been reported, confirming a previously suspected association. One study conducted in the United Kingdom found, as expected, that the greater the number of underlying risk factors for coronary artery disease (cigarette smoking, hypertension, hypercholesterolemia, obesity, diabetes, history of pre-eclampsia toxemia) the higher the risk of developing myocardial infarction, regardless of whether the patient was an O.C. user or not. O.C.s, however, were found to be an additional risk factor. In terms of relative risk, it has been estimated that O.C. users who do not smoke (smoking is considered a major predisposing condition to myocardial infarction) are about twice as likely to have a fatal myocardial infarction as nonusers who do not smoke. O.C. users who are also smokers have about a 5-fold increased risk of fatal infarction compared to users who do not smoke, and about a 10- to 12-fold increased risk compared to nonusers who do not smoke. Furthermore, the number of cigarettes smoked is also an important factor. In determining the importance of these relative risks, however, the baseline rates for various age groups must be given serious consideration. (The estimate of relative risk for British vital statistics which show acute myocardial infarction death rates 2- to 3-times less than in the U.S. for women in these age groups; consequently, actual U.S. death rates could be higher.) The importance of other predisposing conditions mentioned above in determining relative and absolute risks has not been quantified; other synergistic actions may exist. **RISK OF DOSE:** In an analysis of data derived from several national adverse reaction reporting systems, British investigators concluded that the risk of thromboembolism including coronary thrombosis is directly related to the dose of estrogen used in O.C.s. Preparations containing 0.1 mg or more of estrogen were associated with a higher risk of thromboembolism than those containing 0.05-0.08 mg of estrogen. Their analysis did suggest, however, that the quantity of estrogen may not be the sole factor involved. This finding has been supported by a study in the United States. Careful epidemiological studies to determine the degree of thromboembolic risk associated with progestogen-only O.C.s have not been performed. Cases of thromboembolic disease have been reported in women using these products, and they should not be presumed to be free of excess risk. **ESTIMATE OF EXCESS MORTALITY FROM CIRCULATORY DISEASE:** A large British prospective study estimated the mortality rate per 100,000 women per year from diseases of the circulatory system for users and nonusers of O.C.s according to age, smoking habits, and duration of use. The overall excess death rate annually from circulatory diseases for O.C. users was estimated to be 20 per 100,000 (ages 15-34—5/100,000; ages 35-44—33/100,000; ages 45-49—140/100,000). The risk is concentrated in older women, in those with a long duration of use, and in cigarette smokers, and may persist after discontinuation of O.C. use. It was not possible, however, to examine the interrelationships of age, smoking, and duration of use, nor to compare the effects of continuous vs. intermittent use. Although the study showed a 10-fold increase in death due to circulatory diseases in users for 5 or more years, all of these deaths occurred in women 35 or older. Until larger numbers of women under 35 with continuous use for 5 or more years are available, it is not possible to assess the magnitude of the relative risk for this younger age group. The available data from a variety of sources have been analyzed to estimate the risk of death associated with various methods of contraception. The estimates of risk of death for each method include the combined risk of the contraceptive method (e.g., thromboembolic and thrombotic disease in the case of O.C.s) plus the risk attributable to pregnancy or abortion in the event of method failure. The findings of this analysis are shown in Table 1 below. The study concluded that the mortality associated with all methods of birth control is low and below that of childbirth, with the exception of O.C.s in women over 40 who smoke. (The rates given for pill only/smokers for each age group are for smokers as a class. For "heavy" smokers (more than 15 cigarettes a day), the rates given would be about double; for "light" smokers (less than 15 cigarettes a day), about 50 percent.) The lowest mortality is associated with the condom or diaphragm backed up by early abortion. The study also concluded that O.C. users who smoke, especially those over the age of 30, have a greater mortality risk than O.C. users who do not smoke.

**Table 1. The annual number of deaths associated with control of fertility and no control per 100,000 nonsterile women by regimen of control and age of women are:**

|                                        | 15-19 | 20-24 | 25-29 | 30-34 | 35-39 | 40-44 |
|----------------------------------------|-------|-------|-------|-------|-------|-------|
| • No method                            | 5.6   | 6.1   | 7.4   | 13.9  | 20.8  | 22.6  |
| Abortion only                          | 1.2   | 1.6   | 1.8   | 1.7   | 1.9   | 1.2   |
| Pill only—non-smokers                  | 1.3   | 1.4   | 1.4   | 2.2   | 4.5   | 7.1   |
| Pill only—smokers                      | 1.5   | 1.6   | 1.6   | 10.8  | 13.4  | 58.9  |
| IUD only                               | 0.9   | 1.0   | 1.2   | 1.4   | 2.0   | 1.9   |
| Traditional contraception only         | 1.1   | 1.6   | 2.0   | 3.6   | 5.0   | 4.2   |
| Traditional contraception and abortion | 0.2   | 0.2   | 0.3   | 0.3   | 0.3   | 0.2   |

The risk of thromboembolic and thrombotic disease associated with O.C.s increases with age and after approximately age 30 and, for myocardial infarction, is further increased by hypertension, hyperlipidemias, obesity, diabetes, or history of pre-eclampsia toxemia and especially by cigarette smoking. Based on

the data currently available, the following table gives a gross estimate of the risk of death from circulatory disorders associated with the use of O.C.s:

**Table 2. Smoking habits and other predisposing conditions—risk associated with use of O.C.s**

|                                 | Age | Below 30 | 30-39 | 40+ |
|---------------------------------|-----|----------|-------|-----|
| Heavy smokers                   |     | C        | B     | A   |
| Light smokers                   |     | D        | C     | B   |
| Non-smokers                     |     |          | C,D   | C   |
| (No predisposing conditions)    |     | D        |       |     |
| Non-smokers                     |     |          | C,B   | B,A |
| (Other predisposing conditions) |     |          |       |     |

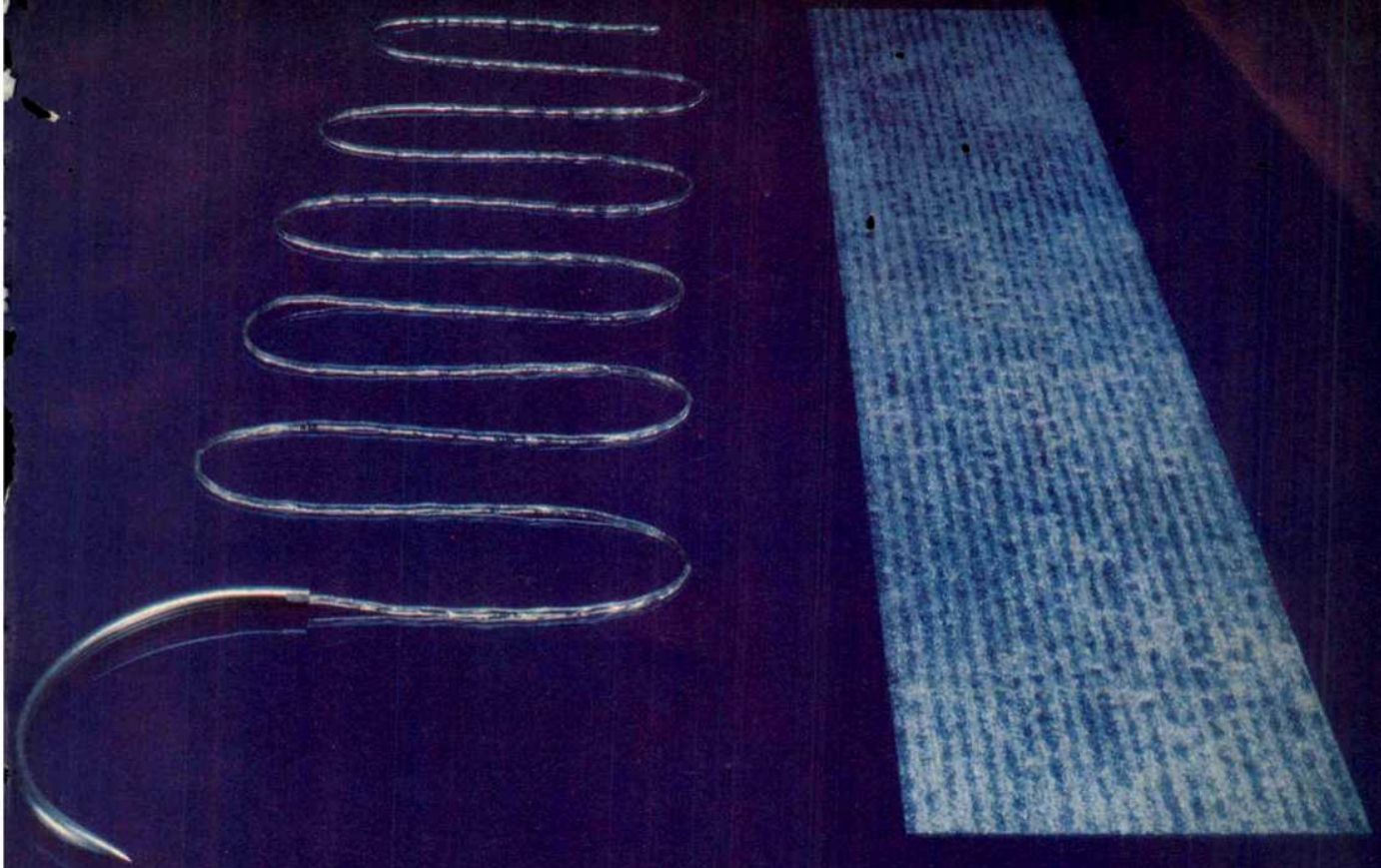
A—Use associated with very high risk. C—Use associated with moderate risk.  
B—Use associated with high risk. D—Use associated with low risk.

The physician and the patient should be alert to the earliest manifestations of thromboembolic and thrombotic disorders (e.g., thrombophlebitis, pulmonary embolism, cerebrovascular insufficiency, coronary occlusion, retinal thrombosis, and mesenteric thrombosis). Should any of these occur or be suspected, the drug should be discontinued immediately. A four- to six-fold increase in risk of post-surgical thromboembolic complications has been reported in O.C. users. If feasible, O.C.s should be discontinued at least 4 weeks before surgery of a type associated with an increased risk of thromboembolism or prolonged immobilization. Data also suggest that the presence of varicose veins substantially increases the risk of superficial venous thrombosis of the leg, the risk depending on the severity of the varicosities. **Ocular Lesions:** There have been reports of neuro-ocular lesions such as optic neuritis or retinal thrombosis associated with the use of O.C.s. Discontinue O.C. medication if there is unexplained, sudden or gradual, partial or complete loss of vision; onset of proptosis or diplopia; papilledema; or retinal vascular lesions; and institute appropriate diagnostic and therapeutic measures. **Carcinoma:** Long-term continuous administration of either natural or synthetic estrogen in certain animal species increases the frequency of certain adenomas, benign or malignant, such as those of the breast, cervix, vagina, uterus, ovary, pituitary and liver. Certain synthetic progestogens, none currently contained in O.C.s, have been noted to increase the incidence of mammary nodules, benign and malignant, in dogs. Several retrospective case-control studies have reported an increased relative risk (3.1 to 13.5 times) associating endometrial carcinoma with the prolonged use of estrogens in postmenopausal women who took estrogen replacement medication to relieve menopausal symptoms. One publication reported on the first 30 cases submitted by physicians to a registry of cases of adenocarcinoma of the endometrium in women under 40 on O.C.s. Of the adenocarcinomas found in women without predisposing risk factors for adenocarcinoma of the endometrium (e.g., irregular bleeding at the time O.C.s were first given, polycystic ovaries) nearly all occurred in women who had used a sequential O.C. These products are no longer marketed. No statistical association has been reported suggesting an increased risk of endometrial cancer in users of conventional combination or progestogen-only O.C.s, although individual cases have been reported. Several studies have shown no increased risk of breast cancer to women taking O.C.s or estrogens. In one study, however, while no overall increased risk of breast cancer was noted in women treated with O.C.s, a greater risk was suggested for the subgroups of O.C. users with documented benign breast disease and for long-term (2-4 years) users. One other study indicated an increasing risk of breast cancer in women taking menopausal estrogens, which increased with duration of follow-up. A reduced occurrence of benign breast tumors in users of O.C.s has been well documented in a prospective study of women with cervical dysplasia, there was an increase in severity and of conversion to cancer *in situ* in O.C. users compared with nonusers. This became statistically significant after 3 to 4 years of use. Nonreversal of dysplasia within the first 6 months of pill use was suggested to be predictive of progression after prolonged exposure. There have been other reports of microglandular hyperplasia of the cervix in users of O.C.s. In summary, there is at present no confirmed evidence from human studies of an increased risk of cancer associated with O.C.s. Close clinical surveillance of all women taking O.C.s is, nevertheless, essential. In all cases of undiagnosed persistent or recurrent abnormal vaginal bleeding, appropriate diagnostic measures should be taken to rule out malignancy. Women with a strong family history of breast cancer or who have breast nodules, fibrocystic disease or abnormal mammograms should be monitored with particular care if they elect to use O.C.s instead of other methods of contraception. **4. Liver Tumors:** Sudden severe abdominal pain or shock may be due to rupture and hemorrhage of a liver tumor. There have been reports associating benign or malignant liver tumors with O.C. use. This has been reported in short-term as well as long-term users of O.C.s. One study reported that use of O.C.s with high hormonal potency and age over 30 years may further increase a woman's risk of hepatocellular adenoma. Two studies relate risk with duration of use, the risk being much greater after 4 or more years of use. Although it is an uncommon lesion, it should be considered in women presenting with an "acute abdomen". The tumor may cause serious or fatal hemorrhage. Patients with liver tumors have demonstrated variable clinical features which may make preoperative diagnosis difficult. Some cases presented because of right upper quadrant masses, while most had signs and symptoms of acute intraperitoneal hemorrhage. Routine radiological and laboratory studies may not be helpful. Liver scans may clearly show a focal defect. Hepatic arteriography may be a useful procedure in diagnosing primary liver neoplasms. **5. Use of or Immediately Preceding Pregnancy, Birth Defects in Offspring, and Malignancy in Female Offspring:** The use of female sex hormones—both estrogenic and progestational agents—during early pregnancy may seriously damage the offspring. It has been shown that females exposed *in utero* to diethylstilbestrol, a nonsteroidal estrogen, have an increased risk of developing in later life a form of vaginal or cervical cancer that is ordinarily extremely rare. This risk has been estimated to be of the order of 1 in 1,000 exposures or less. Although there is no evidence at the present time that O.C.s further enhance the risk of developing this type of malignancy, such patients should be monitored with particular care if they elect to use O.C.s instead of other methods of contraception. Furthermore, a high percentage of women exposed to diethylstilbestrol (from 30 to 90%) have been found to have epithelial changes of the vagina and cervix. Although these changes are histologically benign, it is not known whether this condition is a precursor of vaginal malignancy. Male children so exposed may develop abnormalities of the urogenital tract. Although similar data are not available with the use of other estrogens, it cannot be presumed that they would not induce similar changes. An increased risk of congenital anomalies, including heart defects and limb defects, has been reported following use of sex hormones, including O.C.s, in pregnancy. In one case-control study it was estimated that there was a 4.7-fold increased relative risk of limb-reduction defects in infants exposed *in utero* to sex hormones (O.C.s, hormonal withdrawal tests for pregnancy or attempted treatment for threatened abortion). Some of these exposures were very short and involved only a few days of treatment. The data suggest that the risk of limb-reduction defects in exposed fetuses is somewhat less than 1 in 1,000 live births. In a large prospective study, cardiovascular defects in children born to women who received female hormones (including O.C.s) during early pregnancy occurred at a rate of 18.2 per 1,000 births, compared to 7.6 per 1,000 for children not so exposed *in utero*. These results are statistically significant. In the past, female sex hormones have been used during pregnancy in an attempt to treat threatened or habitual abortion. There is considerable evidence that estrogens are ineffective for these indications, and there is no evidence from well controlled studies that progestogens are effective for these uses. There is some evidence that triploidy and possibly other types of polyploidy are increased among abortuses from women who use O.C.s. O.C.s are contraindicated in women with known or suspected pregnancy. Pregnant women who are virgins should always abort spontaneously. Whether there is an overall increase in spontaneous abortion of pregnancies conceived soon after stopping O.C.s is unknown. If the patient has not adhered to the prescribed schedule, the possibility of pregnancy should be considered at the time of the first missed period (or after 45 days from the last menstrual period if progestogen-only O.C.s are used) and further use of O.C.s should be withheld until pregnancy has been ruled out. It is recommended that for any patient who has missed two consecutive periods, pregnancy should be ruled out before continuing the contraceptive regimen. If pregnancy is confirmed, the patient should be apprised of the potential risks to the fetus and the advisability of continuation of the pregnancy should be discussed in the light of these risks. It is also recommended that women who discontinue O.C.s with the intent of becoming pregnant use an alternate form of contraception for a period of time before attempting to conceive. Many clinicians recommend 3 months, although no precise information is available on which to base this recommendation. The administration of progestogen-only or progestogen-estrogen combinations to induce withdrawal bleeding should not be used as a test of

pregnancy. **6. Other Contraindications:** The following are contraindications to the use of O.C.s or estrogens. In one study, an increased risk appeared after 2 years of use and doubled after 4 or 5 years of use. In another study an increased risk was apparent between 6 and 12 months of use. **7. Carbohydrate and Lipid Metabolic Effects:** A decrease in glucose tolerance has been observed in a significant percentage of patients on O.C.s. For this reason, prediabetic and diabetic patients should be carefully observed while receiving O.C.s. An increase in triglycerides and total phospholipids has been observed in patients receiving O.C.s. The clinical significance of this finding is unknown. **8. Elevated Blood Pressure:** An increase in blood pressure has been reported with O.C. use. In some women, hypertension may occur within a few months of beginning O.C. use. In the first year of use, the prevalence of women with hypertension is low in users and may be no higher than that of a comparable group of nonusers. The prevalence increases, however, with longer exposure, and in the fifth year of use it is two and a half to three times the reported prevalence in the first year. And also strongly correlated with the development of hypertension in O.C. users. Women with a history of elevated blood pressure (hypertension), preexisting renal disease, a history of toxemia or elevated blood pressure during pregnancy, a familial tendency to hypertension or its consequences, or a history of excessive weight gain or fluid retention during the menstrual cycle may be more likely to develop elevation of blood pressure when given O.C.s and, therefore, should be monitored closely. Even though elevated blood pressure may remain within the "normal" range, the clinical implications of elevation should not be ignored and close surveillance is indicated, particularly for women with other risk factors for cardiovascular disease or stroke. High blood pressure may or may not persist after discontinuation of the O.C. **9. Headache:** The onset or exacerbation of migraine or development of headache of a new pattern which is recurrent, persistent, or severe, requires discontinuation of O.C.s and evaluation of the cause. **10. Bleeding Irregularities:** Breakthrough bleeding, spotting, and missed menses are frequent reasons for patients discontinuing O.C.s. In breakthrough bleeding, as in all cases of irregular bleeding from the vagina, nonfunctional causes should be borne in mind. In undiagnosed persistent or recurrent abnormal bleeding from the vagina, adequate diagnostic measures are indicated to rule out pregnancy or malignancy. If pathology has been excluded, time or a change to another formulation may solve the problem. Change to a new O.C. with a higher estrogen content, while potentially useful in minimizing menstrual irregularity, should be done only if necessary since this may increase the risk of thromboembolic disease. Women with a past history of oligomenorrhea or secondary amenorrhea or young women without regular cycles may have a tendency to remain anovulatory or to become amenorrheic after discontinuation of O.C.s. Women with these preexisting problems should be advised of this possibility and encouraged to use other contraceptive methods. Post-use anovulation, possibly prolonged, may also occur in women without previous irregularities. A higher incidence of galactorrhea and of pituitary tumors (e.g., adenomas) has been associated with amenorrhea in former users compared with nonusers. One study reported a 16-fold increased prevalence of pituitary prolactin-secreting tumors among patients with postpill amenorrhea when galactorrhea was present. **11. Infertility:** There is evidence of impairment of fertility in women discontinuing O.C.s in comparison with those discontinuing other methods of contraception. The impairment appears to be independent of the duration of use of the preparations. While the impairment diminishes with time, there is still an appreciable difference in the results in nulliparous women for the O.C. and non-O.C. groups 30 months after discontinuation of birth control. For parous women the difference is no longer apparent 30 months after cessation of contraception. **12. Ectopic Pregnancy:** Ectopic as well as intrauterine pregnancy may occur in contraceptive failures. However, in progestogen-only O.C. failures, the ratio of ectopic to intrauterine pregnancies is higher than in women who are not receiving O.C.s, since the drugs are more effective in preventing intrauterine than ectopic pregnancies. **13. Breast Feeding:** O.C.s given in the postpartum period may interfere with lactation. There may be a decrease in the quantity and quality of the breast milk. Furthermore, a small fraction of the hormonal agents in O.C.s has been identified in the milk of mothers receiving these products. Thus, if any cases on the breast fed child have not been determined. If feasible, the use of O.C.s should be deferred until the infant has been weaned. **PRECAUTIONS GENERAL:** 1. A complete medical and family history should be taken prior to the initiation of O.C.s. The pretreatment and periodic physical examinations should include special reference to blood pressure, breasts, abdomen and pelvic organs, including Papanicolaou smear and relevant laboratory tests. As a general rule, O.C.s should not be prescribed for longer than 1 year without another physical examination being performed. 2. Under the influence of estrogen-progestogen O.C.s, there may be preexisting uterine leiomyomata may increase in size. 3. Patients with a history of psychic depression should be carefully observed and the drug discontinued if depression recurs to a serious degree. Patients becoming significantly depressed while taking O.C.s should stop the medication and use an alternate method of contraception in an attempt to determine whether the symptom is drug related. 4. O.C.s may cause some degree of fluid retention. They should be prescribed with caution, and only with careful monitoring, in patients with conditions which might be aggravated by fluid retention, such as convulsive disorders, migraine syndrome, asthma or cardiac, hepatic or renal insufficiency. 5. Patients with a past history of jaundice during pregnancy have an increased risk of recurrence of jaundice while receiving O.C. therapy. If jaundice develops in any patient receiving such drugs, the medication should be discontinued. 6. Steroid hormones may be poorly metabolized in patients with impaired liver function and should be administered with caution in such patients. 7. O.C. users may have disturbances in normal tryptophan metabolism which may result in a relative pyridoxine deficiency. The clinical significance of this is unknown. 8. Serum folate levels may be depressed by O.C. therapy. Since the pregnant woman is predisposed to the development of folate deficiency and the incidence of the mild deficiency increases with increasing gestation, it is possible that if a woman becomes pregnant shortly after stopping O.C.s, she may have a greater chance of developing folate deficiency and complications attributed to this deficiency. 9. The pathologist should be advised of O.C. therapy when relevant specimens are submitted. 10. Certain endocrine and liver function tests and blood components may be affected by estrogen-containing O.C.s. For example: a. Increased sulfobromophthalen retention. b. Increased prothrombin and factors VII, VIII, IX and X; decreased antithrombin III; increased norepinephrine-induced platelet aggregability. c. Increased thyroid binding globulin (TBG) leading to increased circulating thyroid hormone, as measured by protein-bound iodine (PBI), T4 by column, or T4 by radioimmunoassay. Free T3 resin uptake is decreased, reflecting the elevated TBG. Free T4 concentration is unaltered. d. Decreased pregnandiol excretion. e. Reduced response to metyrapone test. **DRUG INTERACTIONS:** O.C.s may be rendered less effective by virtue of drug interaction with rifampin, isoniazid, ampicillin, tetracycline, neomycin, penicillin V, chloramphenicol, sulfonamides, nitrofurantoin, barbiturates, phenytoin, primidone, analgesics, tranquilizers, and antigravitate preparations. O.C.s may alter the effectiveness of other types of drugs, such as oral anticoagulants, anticonvulsants, tricyclic antidepressants, antihypertensive agents (e.g., guanethidine), vitamins and hypoglycemic agents. **CARCINOGENESIS:** See Warnings. **PREGNANCY:** Pregnancy category X. See Contraindications and Warnings. **NURSING MOTHERS:** See Warnings. **Adverse Reactions:** An increased risk of the following serious adverse reactions has been associated with the use of O.C.s (see Warnings): thrombophlebitis, thrombosis, pulmonary embolism, coronary thrombosis, cerebral thrombosis, mesenteric thrombosis, liver tumors, cerebral hemorrhage, hypertension, gall bladder disease, congenital anomalies, neuro-ocular lesions, e.g., retinal thrombosis and stroke. The following adverse reactions have been reported in patients receiving O.C.s and are believed to be drug related: bleeding irregularities (breakthrough bleeding, spotting, missed menses during treatment, amenorrhea after treatment), gastrointestinal symptoms (nausea, vomiting, bloating, abdominal cramps), dysmenorrhea, infertility after discontinuance of treatment, edema, chloasma or melasma which may persist after drug is discontinued, breast changes (tenderness, enlargement, and pain), decreased tolerance to contact lenses, change in corneal curvature, change in vision (increase or decrease), change in hair growth (steepening) change in weight (increase or decrease), change in cervical erosion and cervical secretions, cholestatic jaundice, migraine, increase in size of uterine leiomyomata, rash (allergic), mental depression, reduced tolerance to carbohydrates, vaginal candidiasis, prolatin-secreting pituitary tumors. The following adverse reactions have been reported in users of O.C.s, and the association has been neither confirmed nor refuted: premenstrual-like syndrome, cataracts, changes in libido, chorea, changes in appetite, cystitis-like syndrome, headache, nervousness, dizziness, hirsutism, loss of hair, erythema, multifactorial, erythema nodosum, hemorrhagic eruption, edema, leukorrhea, porphyria, impaired renal function. **Information for the Patient (See Patient Package Insert)**



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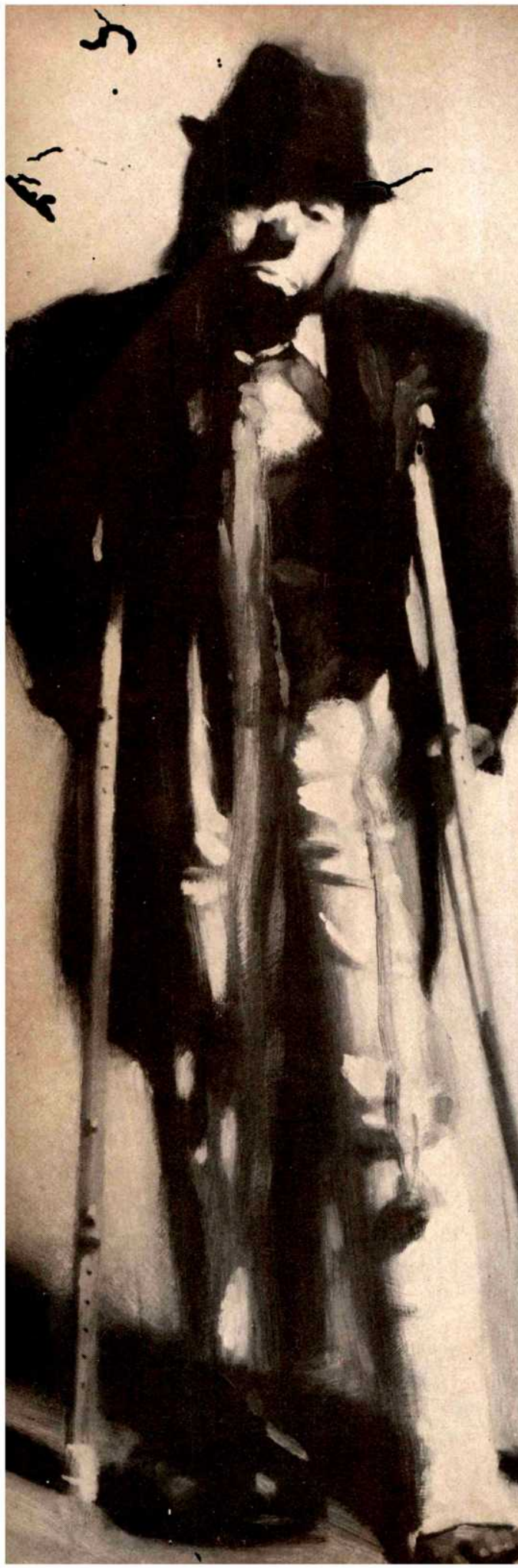
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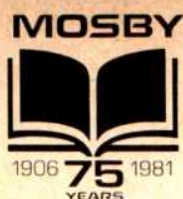
**DOSAGE AND ADMINISTRATION:** Dosage should be adjusted according to the severity of the pain and the response of the patient. It may occasionally be necessary to exceed the usual dosage recommended below in cases of more severe pain or in those patients who have become tolerant to the analgesic effect of narcotics. Empirin with Codeine is given orally. The usual adult dose for Empirin with Codeine No. 2 and No. 3 is one or two tablets every four hours as required. The usual adult dose for Empirin with Codeine No. 4 is one tablet every four hours as required.

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# American Journal of Obstetrics and Gynecology

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## Contents

January 1

1981

## Obstetrics

- Pregnancy complicated by maternal heart disease at the National Maternity Hospital, Dublin, Ireland, 1969 to 1978** 1  
Declan Sugrue, M.B., M.R.C.P., M.R.C.P.I., Sean Blake, M.D., M.R.C.P., F.R.C.P.I., and Dermot MacDonald, M.B., M.A.O., F.R.C.O.G.  
*Dublin, Ireland*
- The nonstress test: A review of 3,000 tests** 7  
Jeffrey P. Phelan, Lieutenant Commander, MC, USNR  
*Portsmouth, Virginia*
- Pregnancy outcome in the patient with a nonreactive nonstress test and a positive contraction stress test** 11  
Charles Slomka, Lieutenant, MC, USNR, and  
Jeffrey P. Phelan, Lieutenant Commander, MC, USNR  
*Portsmouth, Virginia*

(Contents continued on page 7)

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## Fetus, placenta, and newborn

---

- Naloxone and its possible relationship to fetal endorphin levels and fetal distress** 16

Robert C. Goodlin, M.D.

*Davis, California, and Omaha, Nebraska*

- Maternal and fetal concentrations of morphine after epidural administration during labor** 20

Gunilla Nybell-Lindahl, M.D., Christer Carlsson, M.D., Ingemar Ingemarsson, M.D.,

Magnus Westgren, M.D., and Lennart Paalzow, Ph.D.

*Lund and Solna, Sweden*

- Effect of endogenous insulin release on fetal alanine concentration and uptake** 22

Anthony F. Philipps, Joseph W. Dubin, and John R. Raye

*Farmington, Connecticut*

- Fetal macrosomia in experimental maternal diabetes** 27

Yee S. Kim, Ph.D., Yang-Ja Yoon, B.S., Ismail Jatoi, B.S., and Young Kim, Ph.D.

*St. Louis, Missouri*

- Short-term variability of fetal heart rate during pregnancies with normal and insufficient placental function** 33

Veikko Kariniemi and Pirkko Ämmälä

*Helsinki, Finland*

- Maternal and cord blood complement activity: Relationship to premature rupture of the membranes** 38

Donald L. Levy, M.D., and Pierre C. Arquembourg, M.D.

*New Orleans, Louisiana*

- Protein synthesis by human fetal lung** 41

O. Genbačev, B. Čemerikić, M. Movsesijan, and V. Šulović

*Banatska, Yugoslavia*

---

## Gynecology

---

- Early menopause and the risk of myocardial infarction** 47

Lynn Rosenberg, Sc.D., Charles H. Hennekens, M.D., Bernard Rosner, Ph.D.,

Charlene Belanger, M.A., Kenneth J. Rothman, Dr.P.H., and Frank E. Speizer, M.D.

*Boston, Massachusetts*

- Oral contraceptive use, sexual activity, and cervical carcinoma** 52

Shanna H. Swan, Ph.D., and Willard L. Brown, M.D., M.P.H.

*Walnut Creek, California*

(Contents continued on page 9)



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## Contents continued from page 7

|                                                                                                                                                                                                                                  |    |
|----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|----|
| <b>Premenopausal hysterectomy and cardiovascular disease</b>                                                                                                                                                                     | 58 |
| Brandon S. Centerwall, M.D., M.P.H.<br><i>New Orleans, Louisiana</i>                                                                                                                                                             |    |
| <b>Ovarian failure in long-term survivors of childhood malignancy</b>                                                                                                                                                            | 62 |
| Robert J. Stillman, M.D., Jay S. Schinfeld, M.D., Isaac Schiff, M.D.,<br>Richard D. Gelber, Ph.D., Joel Greenberger, M.D., Martin Larson, M.S., Norman Jaffe, M.D.,<br>and Frederick P. Li, M.D.<br><i>Boston, Massachusetts</i> |    |
| <b>Modulation of protein expression in endometrial adenocarcinoma cells by in vitro exposure to estradiol and progesterone</b>                                                                                                   | 67 |
| T. J. O'Brien, W. Hernandez, P. J. Jernstrom, D. B. Seymour, C. P. Morrow, and J. A. Sykes<br><i>Los Angeles, California</i>                                                                                                     |    |
| <b>Reversal of female sterilization: Comparison of microsurgical and gross surgical techniques for tubal anastomosis</b>                                                                                                         | 73 |
| Simon R. Henderson, M.D., D.Phil.<br><i>San Francisco, California</i>                                                                                                                                                            |    |
| <b>Intestinal operations in patients with ovarian carcinoma</b>                                                                                                                                                                  | 80 |
| Thomas W. Castaldo, M.D., Edmund S. Petrilli, M.D., Samuel C. Ballon, M.D., and<br>Leo D. Lagasse, M.D.<br><i>Los Angeles and Duarte, California</i>                                                                             |    |

## Current developments

|                                                                |    |
|----------------------------------------------------------------|----|
| <b>Premenstrual syndrome</b>                                   | 85 |
| Robert L. Reid and S. S. C. Yen<br><i>La Jolla, California</i> |    |

## Communications in brief

|                                                                                                             |     |
|-------------------------------------------------------------------------------------------------------------|-----|
| <b>Endometriosis in a patient with Rokitansky-Kuster-Hauser syndrome</b>                                    | 105 |
| David L. Rosenfeld, M.D., and B. Douglas Lecher, M.D.<br><i>Manhasset, New York</i>                         |     |
| <b>Culture-proved Fitz-Hugh-Curtis syndrome</b>                                                             | 106 |
| Stephen J. Kornfeld, M.D., and Michael G. Worthington, M.D.<br><i>Boston and Brighton, Massachusetts</i>    |     |
| <b>Correlation of daily fetal movements and the nonstress test as tools for assessment of fetal welfare</b> | 107 |
| James A. O'Leary, M.D., and George C. Andrinopoulos, M.D.<br><i>Mobile, Alabama</i>                         |     |

(Contents continued on page 11)



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## Contents continued from page 9

---

- Recovery of *Neisseria meningitidis* from the cul-de-sac of a woman with endometritis-salpingitis-peritonitis** 108

Gilles R. G. Monif, M.D.  
*Gainesville, Florida*

- Paravestibular tumor of the female genital tract** 109

Wesley C. Fowler, Jr., M.D., F.A.C.O.G., Hal Lawrence, M.D., and  
David A. Edelman, Ph.D.  
*Chapel Hill, North Carolina*

- Prenatal diagnosis of diastrophic dwarfism** 111

Stephanos Mantagos, M.D., Robert R. Weiss, M.D., Maurice Mahoney, M.D., and  
John C. Hobbins, M.D.  
*East Meadow, New York, and New Haven, Connecticut*

## Correspondence

---

- Correspondence** 114

## Roster

---

- Roster of American obstetrical and gynecological societies** 118

## Items

---

- Items** 121

*Information for authors on page 21*

*Index to advertisers on page 40*

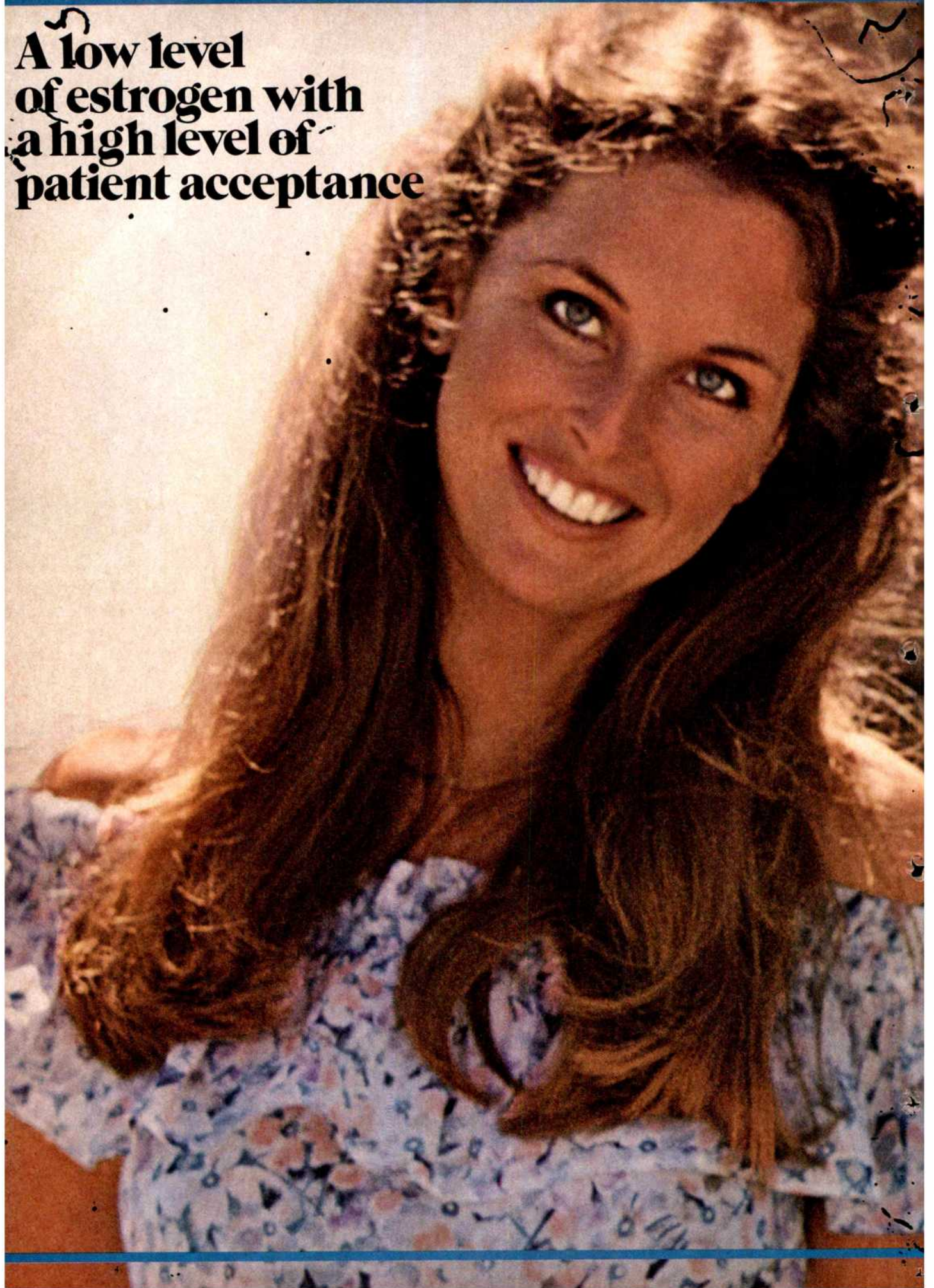
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**Contraindications**—Thrombophlebitis, thromboembolic disorders, cerebrovascular disease, myocardial infarction or coronary artery disease, or past history of these, known or suspected breast carcinoma, estrogen-dependent neoplasia, or pregnancy, undiagnosed abnormal vaginal bleeding, markedly impaired liver function, past or present, benign or malignant liver tumors.

## Warnings

**Cigarette-smoking increases risk of serious cardiovascular side effects from OC use. Risk increases with age and heavy smoking (15 or more cigarettes/day) and is quite marked in women over 35. OC users should be strongly advised not to smoke.**

OCs are associated with increased risk of several serious conditions including venous and arterial thromboembolism, thrombotic and hemorrhagic stroke, myocardial infarction, visual disorders, hepatic tumor, gallbladder disease, hypertension, fetal abnormalities. Practitioners should be familiar with the available information relating to these and other risks.

**1. Thromboembolic disorders and other vascular problems**—Increased risk of thromboembolic and thrombotic disease associated with OCs is well established. A British study showed increased relative risk for fatal venous thromboembolism; 2 British and 3 U.S. studies showed increased relative risk for nonfatal venous thromboembolism. Two U.S. studies showed increased relative risk for stroke, not shown in prior British studies. It was estimated OC users were 2–11 times more likely than nonusers to manifest these diseases without evident cause. In a British study of idiopathic deep vein thrombosis and pulmonary embolism, projected hospitalization rates for ages 16–40 were 47/year/100,000 users and 5 for nonusers. In a British mortality study, overall excess mortality from pulmonary embolism or stroke was 1.3–3.4 deaths/year/100,000 users and increased with age. **Cerebrovascular Disorders**—In a U.S. study of stroke with and without predisposing causes, risk of hemorrhagic stroke was estimated as 2 times greater and thrombotic stroke 4.0–9.5 times greater in users. Mortality trends in 21 countries indicate that, since OC introduction, changes in mortality from nonhemorrhagic cerebrovascular disease were associated with changes in the prevalence of OC use. The Royal College of GPs reported on its prospective study comparing users and nonusers. "A statistically significant higher rate of reporting of cerebrovascular accidents in takers is evident, but the numbers are too small to justify an estimation of the degree of risk." They reported more superficial and deep vein thrombosis in users. A 1977 analysis showed a 5-fold increased mortality in users from circulatory diseases, mainly myocardial infarction (MI) and hemorrhagic stroke. Excess mortality was associated with age, smoking and duration of use. In 1976 a British Family Planning Association study found a highly significant association between OC use and stroke though total numbers were small; the increased risk for venous thrombosis and pulmonary embolism was about 4-fold and statistically highly significant. Circulatory disease mortality was significantly greater in users. **Myocardial Infarction**—An increased risk of MI associated with OC use was reported in Britain, as previously suspected. The morbidity study found the greater the number of risk factors for coronary artery disease (smoking, hypertension, hypercholesterolemia, obesity, diabetes, history of pre-eclamptic toxemia) the higher the MI risk, regardless of OC use. OCs were considered an additional risk factor. The excess rate of fatal MI in Britain was about 3.5/year/100,000 users aged 30–39 and 20 for ages 40–44. (These are based on British vital statistics with acute MI death rates 2–3 times less than the U.S. Similar estimates for U.S. are 257 for users aged 30–39 with predispositions vs 157 without; for ages 40–44, 86.2 vs 5.1.) Excess hospitalization rate for nonfatal MI in Britain was about 3.5/year/100,000 users aged 30–39 and 47 for ages 40–44. Smoking is a major predisposing condition to MI. In terms of relative risk, nonsmoking OC users are twice as likely to have fatal MI as nonsmoking nonusers. Smoking users have a 5-fold greater risk of fatal MI as nonsmoking users, but a 10–12-fold greater risk than nonsmoking nonusers. Amount of smoking is an important factor. In determining importance of these risks, give serious consideration to rates in table below. Importance of other predispositions cited above in determining relative and absolute risks has not been quantified; it is likely the same synergism exists, but perhaps to a lesser extent.

Estimated Mortality Rate from Myocardial Infarction per 100,000 Women/Year

|                            | Aged 30–39 |          | Aged 40–44 |          |
|----------------------------|------------|----------|------------|----------|
|                            | Users      | Nonusers | Users      | Nonusers |
| All smokers                | 10.2       | 2.6      | 62.0       | 15.9     |
| $\geq 15$ cigarettes daily | 13.0       | 5.1      | 78.7       | 31.3     |
| $<15$ cigarettes daily     | 4.7        | 0.9      | 28.6       | 5.7      |
| Nonsmokers                 | 1.8        | 1.2      | 10.7       | 7.4      |
| Smokers and nonsmokers     | 5.4        | 1.9      | 32.8       | 11.7     |

**Risk of Dose**—Reports of thromboembolism after use of OCs with  $\geq 50$  mcg estrogen received by drug safety committees in Britain, Sweden and Denmark were compared with the distribution expected from market research sales estimates. A positive correlation was found between estrogen dose and reporting of thromboembolism, including coronary thrombosis, in excess of that predicted by sales. OCs with  $\geq 100$  mcg estrogen were associated with higher thromboembolic risk than those with 50–80 mcg. The authors suggested that estrogen quantity may not be the sole factor. Any influence of progestogens was not considered, which may be responsible for certain discrepancies in the data. No significant differences were noted between OCs containing the same estrogen dose but between ethinyl estradiol and mestranol. Studies on thromboembolic risk with progestogen-only OCs have not been done. Cases have been reported for these OCs and they are not presumed free of excess risk. In a U.S. case-controlled study, relative risk of OC use 1 month before hospitalization for various types of thromboembolism was calculated. If relative estrogenic potency and possible influence of different progestogens are ignored, OCs may be divided into those with  $<100$  mcg, and those with  $\geq 100$  mcg estrogen. For all cases combined, larger doses were associated with only slightly higher relative risk, for idiopathic cases relative risk was doubled with larger doses, but confidence limits overlapped and differences were not significant. Apparently there was no increased risk for cases with predispositions. Thromboembolic risk in users and nonusers increases with age. OCs have been considered an independent risk factor. **Excess Mortality from Circulatory Diseases**—A U.K. prospective study estimated annual mortality from circulatory diseases for OC users and nonusers according to age, smoking habits and duration of use. Excess mortality was 20,000/100,000 users (5 for ages 15–34, 33 for ages 35–44, 140 for ages 45–49), the risk being concentrated in older women, long-term users, and smokers. Interrelationships of age, smoking and duration of use could not be examined, nor could effects of continuous vs. intermittent use. Though circulatory mortality was increased 10-fold in users of  $>5$  years, all deaths were in women over age 34. Until more under-35 users of 5+ continuous years are available, size of relative risk for this age group cannot be given. Risk of circulatory disease may have persisted after discontinuance of OCs. One study estimated risk of death with various contraceptive methods, each estimate including risk of method (e.g., thromboembolism for OCs) plus risk due to pregnancy or abortion in cases of method failure. This latter risk varies with effectiveness. It concluded mortality with all methods is low and below that of childbirth, except for OC users over 40 who smoke. Heavy smokers have double the risk of smokers as a class, light smokers about half. Mortality associated with OC use in nonsmokers over 40 is higher than with any other method in that age group. Lowest mortality with the condom or diaphragm backed up by early legal abortion. Risk of thromboembolic and thrombotic disease associated with OCs increases with age after 30 and, for MI, is further increased by hypertension, hypercholesterolemia, obesity, diabetes, history of pre-eclamptic toxemia, and especially cigarette smoking. Based on current data, the chart gives gross estimates of risk of death from circulatory disorders associated with OCs.

| Risk is: A—very high, B—high, C—moderate, D—low | Age | $<30$ | 30–39 | $\geq 40$ |
|-------------------------------------------------|-----|-------|-------|-----------|
| Heavy smokers ( $\geq 15$ /day)                 | C   | B     | A     | A         |
| Light smokers ( $<15$ /day)                     | D   | C     | B     | B         |
| Nonsmokers, no predispositions                  | D   | D     | C     | C         |
| Nonsmokers, with predispositions                | C   | C     | B     | B, A      |

Physician and patient should be alert to earliest manifestations of thromboembolic and thrombotic disorders (e.g., thrombophlebitis, pulmonary embolism, cerebrovascular insufficiency, coronary artery disease, myocardial infarction, retinal thrombosis, mesenteric thrombosis). Should any of these occur or be suspected, discontinue OCs immediately. A 4–7-fold increased risk of post-surgery thromboembolism was reported in users; if feasible discontinue OCs at least 4 weeks before elective surgery or during prolonged immobilization and do not resume until at least 4 weeks post-surgery. Thromboembolic risk may be increased for users with blood types A, B or AB. **2. Ocular Lesions**—Neuro-ocular lesions such as optic neuritis or retinal thrombosis have been associated with OCs. Discontinue if there is unexplained, gradual or sudden, partial or complete loss of vision, proptosis or diplopia, papilledema, or evidence of retinal vascular lesions. Institute diagnostic and therapeutic measures. **3. Carcinoma**—Long-term estrogen in certain animals increases carcinomas and/or nonmalignant neoplasms, such as those of breast, uterus, cervix, vagina, ovary, liver, pituitary. Some

synthetic progestogens, none currently in OCs, increase incidence of benign and malignant mammary nodules in dogs. There is now evidence that estrogens increase risk of human endometrial carcinoma. In several case-controlled studies increased relative risk (2.2–13.9 times) associated endometrial carcinoma with prolonged estrogen for menopausal symptoms. Risk was independent of other known risk factors and depended on duration of use and dose. This is supported by increased endometrial cancer incidence since 1969 in U.S. areas with cancer-reporting systems, which may be related to rapidly expanding estrogen use. There is no evidence that natural estrogens are more or less hazardous than synthetics at equi-estrogenic doses. Of the first 30 cases of endometrial carcinoma in OC users under 40, those without predispositions (e.g., irregular bleeding before OCs, polycystic ovaries) occurred nearly always with no longer-marketed sequential OCs. No statistical association is reported suggesting increased endometrial cancer with combination or progestogen-only OCs, though individual cases have been reported. Studies show no increased breast cancer with OCs or estrogens. However, greater risk was noted in OC users with benign breast disease or long-term (2–4 years) use. Risk of breast cancer with menopausal estrogens rose and further increased with duration of follow-up. Decreased benign breast tumors in OC users is well known. Some studies suggested increased risk of cervical dysplasia, erosion and carcinoma in long-term users, cause and effect is not established. Cervical microglandular hyperplasia has been reported. In summary, there is no confirmed evidence from human studies of increased cancer risk with OCs. Close surveillance is, however, essential. In undiagnosed persistent or recurrent abnormal vaginal bleeding, consider nonfunctional causes, and take appropriate diagnostic measures to rule out malignancy. Carefully monitor users with a strong family history of breast cancer or with breast nodules, fibrocystic disease, recurrent cystic mastitis, abnormal mammograms, or cervical dysplasia. **4. Hepatic adenomas, hepatomas, hamartomas, regenerating nodules, focal nodular hyperplasia, hemangiomas, hepatocellular carcinoma, etc.** have been associated with OCs. In a study OC formulations with high "hormonal potency," were associated with higher risk. Though benign hepatic lesions may rupture and cause fatal hemorrhage in short- or long-term users. Two studies related risk to duration of use, being much greater after  $\geq 4$  years. Consider such lesions in users with abdominal pain, tenderness, mass or shock. About 25% present with abdominal masses, up to half with acute hemoperitoneum. Routine radiologic and lab studies may not be helpful, liver scans may show a focal defect. Hepatic arteriography or CAT may be useful in diagnosis. Hepatocellular carcinoma was noted in a few users. OCs are contraindicated in case of past or present, benign or malignant liver tumors. **5. Use in or immediately preceding pregnancy, birth defects, malignancy in offspring**—Estrogens or progestogens during early pregnancy may seriously damage offspring. Females exposed in utero to DES and other nonsteroidal estrogens may have increased risk of vaginal or cervical cancer. Though OCs may not enhance this risk, monitor such patients carefully. From 30–90% of DES-exposed women develop benign vaginal or cervical adenosis, which could be premalignant. Males so exposed may develop urogenital defects. Other estrogens may mimic DES. Reports relate fetal exposure to female sex hormones and congenital anomalies, e.g., multiple anomalies described by VACTERL for vertebral, anal, cardiac, tracheoesophageal, renal and limb defects. There appears preferential expression by males. There was a 4.7-fold increased risk of limb reduction defects in infants exposed in utero to sex hormones (OCs, hormonal withdrawal pregnancy tests or treatment for threatened abortion). Some exposures were very short, being only a few days. Cardiovascular defects in children exposed to female hormones, including OCs, during early pregnancy occurred at 18,200,000 births, compared to 7.8 for those unexposed (P < .05). Female sex hormones were used for threatened or habitual abortion, but there is considerable evidence estrogens are ineffective and no evidence progestogens are effective. Triploidy and possibly other polyploidy may be increased in abortions from women becoming pregnant soon post-OC. These almost always abort spontaneously. It is unknown if spontaneous abortion is increased soon after stopping OCs. Safety of OCs in pregnancy has not been demonstrated. Rule out pregnancy in using OCs and always consider it if withdrawal bleeding does not occur. If 2 consecutive periods are missed rule out pregnancy before continuing. If user deviates from regimen, consider pregnancy at first missed period and withhold OCs. If pregnancy is confirmed, apprise patient of potential fetal risks and discuss continuation of pregnancy. Users discontinuing for pregnancy should use alternative contraception for 3 months before attempting to conceive, though this is not based on precise information. Do not use progestogen-only or progestogen-estrogen drugs as pregnancy tests. **6. Gallbladder disease** risk, surgically confirmed, appeared after 2 years' use and doubled after 4–5 years in 1 study, it appeared in 5–12 months' use in another. **7. Carbohydrate and lipid metabolism**—Decreased glucose tolerance in a significant number of users, carefully observe diabetic and prediabetic patients. Triglycerides and total phospholipids may increase. **8. Elevated blood pressure (BP)** occurs in some users in a few months, and degree may relate to progestogen dose. Hypertension prevalence in users is no higher than in nonusers in the first year, but increases with longer exposure; in the 5th year it is 2½–3 times the first year. Age is strongly correlated with hypertension in users. Those with history of elevated BP (hypertension), pre-existing renal disease, history of toxemia or elevated BP in pregnancy, familial tendency to hypertension or its consequences, or history of excessive weight gain or fluid retention during menstrual cycles may be more likely to develop elevated BP on OCs and thus should be monitored closely. Even though elevated BP may be in the "normal" range, do not ignore clinical implications. Follow closely, particularly those with other risk factors for cardiovascular disease or stroke. High BP may or may not persist after stopping OCs. **9. Headache**—Onset or exacerbation of migraine or development of headache of a new pattern that is recurrent, persistent or severe requires stopping OCs and evaluating cause. **10. Bleeding irregularities**—Breakthrough bleeding (BTB), spotting and amenorrhea are frequent reasons for discontinuing. In BTB, as in all cases of irregular bleeding, consider nonfunctional causes. In undiagnosed persistent or recurrent bleeding, rule out pregnancy or irregularity. If pathology is excluded, time or change to another product may suffice. Change to a higher estrogen OC only when necessary as this may increase thromboembolic risk. Those with a history of oligomenorrhea or secondary amenorrhea or without established regular cycles may be anovulatory or amenorrheic after discontinuing. Those with such problems should be so informed and other methods encouraged. Post use anovulation, possibly prolonged, may occur in women without previous irregularities. Higher incidence of galactorrhea and of pituitary tumors (e.g., adenomas) has been associated with postpill amenorrhea. One study reported 16-fold increased prevalence of pituitary prolactin-secreting tumors in patients with postpill amenorrhea when galactorrhea was present. **11. Estropic**—In failures with combination OCs, the ratio of ectopic to intrauterine pregnancies is no higher than in nonusers. There may be an increased risk of ectopics after discontinuing. **12. Breast feeding**—OCs may decrease quantity and quality of milk. A small fraction of OC steroids appears in milk. Effects if any on the child are unknown. If feasible defer OCs until after weaning. **13. Infertility** may occur in women discontinuing OCs, which appears independent of duration of use. While impairment diminishes with time, there is still an appreciable difference in nulliparous but not parous women after 30 months; difference is negligible after 42 months.

**Precautions**—Conduct complete history and physical prior to OC use and annually thereafter. Include breasts, abdomen and pelvis; with BP pap and lab tests. Pre-existing uterine leiomyomata may enlarge. Mental depression may occur, with positive history observe carefully and discontinue if serious recurrence. Fluid retention may occur; prescribe cautiously and monitor carefully in convulsive disorders, migraine, asthma, or cardiac, hepatic or renal dysfunction. History of jaundice in pregnancy increases risk of recurrence on OCs; observe carefully and discontinue if jaundice occurs. Steroids may be poorly metabolized in liver dysfunction and should be given cautiously. Tryptophan metabolism may be disturbed and result in pyridoxine deficiency. Megaloblastic anemia has been reported. Serum folate may be depressed and may be exacerbated in post-OC pregnancy. Advise pathologist of OC use when submitting specimens. Endocrine and liver function tests may be altered; repeat abnormal tests after stopping for 2 months. These have been observed: increased BSP retention and other abnormalities in liver function tests; increased prothrombin and coagulation factors VII, VIII, IX, X; decreased antithrombin III, increased platelet aggregability, increased thyroid-binding globulin leading to increased circulating total thyroid hormone (PBI, T<sub>4</sub>); decreased T<sub>3</sub> uptake, unaltered free T<sub>3</sub>; decreased pregnanediol excretion; reduced methyrapone response; increased blood transaminase, corticosteroid, triglyceride, phospholipid; reduced serum folate; impaired glucose tolerance; altered plasma trace minerals. Influence of prolonged OC use on pituitary, ovarian, adrenal, hepatic, or uterine function, or on immune response is not established. OCs may mask onset of climacteric. **Information for patients**—see patient labeling. **Drug interactions**—OC effectiveness may be decreased and more BTB occur with rifampicin, isoniazid, ampicillin, neomycin, penicillin V, chloramphenicol, sulfonamides, nitrofurantoin, barbiturates, phenytoin, primidone, phenylbutazone, analgesics, tranquilizers, antismultrines. OCs may alter effectiveness of drugs such as oral anticoagulants, anticonvulsants, tricyclic antidepressants, antihypertensives, vitamins, hypoglycemics.

**Adverse reactions**—Increased risk of the following serious adverse reactions has been associated with OCs: thrombophlebitis and thrombosis; pulmonary embolism; myocardial infarction and coronary thrombosis; cerebral thrombosis; cerebral hemorrhage; hypertension; gallbladder disease; benign adenomas and other hepatic lesions; with or without intra-abdominal bleeding; congenital abnormalities. There is evidence of association between the following conditions and OCs, though confirmatory studies have not been done: mesenteric thrombosis, Budd-Chiari syndrome, neuro-ocular lesions, e.g., retinal thrombosis and optic neuritis. The following have been reported with OCs and are believed drug related: nausea, vomiting, gastrointestinal symptoms, breakthrough bleeding, spotting, change in menstrual flow, dysmenorrhea, amenorrhea during and after use, infertility post-OC, edema, chloasma or melasma, which may persist post-OC, breast changes (tenderness, enlargement, secretion), weight changes, change in cervical erosion and secretion, endocervical hyperplasia, lactation decrease, cholestatic jaundice, migraine, enlarged uterine leiomyomata, allergic rash, mental depression, reduced carbohydrate tolerance, vaginal candidiasis, change in corneal curvature (steepening), intolerance to contact lenses. The following have been reported and association has not been confirmed or refuted: premenstrual-like syndrome, cataracts, libido changes, chorea, appetite changes, cystitis-like syndrome, headache, paraesthesia, nervousness, dizziness, auditory disturbances, rhinitis, fatigue, backache, hirsutism, scalp hair loss, erythema multiforme or nodosum, hemorrhagic eruption, itching, vaginitis, porphyria, impaired renal function, anemia, pancreatitis, hepatitis, colitis, gingivitis, lupus erythematosus, rheumatoid arthritis, pituitary tumors (e.g., adenoma) with amenorrhea and/or galactorrhea after use, endometrial, cervical and breast carcinoma.

**Acute overdosage**—Serious ill effects have not been reported after large acute doses of OCs in young children, but nausea and withdrawal bleeding might occur.

**Important note**—Complete prescribing information should be consulted prior to use and is available on request.

**SEARLE**

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# When she can't manage, you can.

The sweats. The hot flushes. The distress and discomfort of atrophic vaginitis. The possibility of osteoporosis.\* The menopause.

For many women, it's a time of confusion and frustration. Counseling can help her overcome these feelings. But counseling *can't* stop vasomotor and vaginal symptoms, or retard bone loss\*...the true signs of estrogen deficiency. And that's where she needs you most.

You're an expert at estrogen replacement. You know why it should be used. When it's best avoided. How to cycle therapy. Determine

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When it comes to managing the estrogen-deficiency symptoms of the menopause, you've got what it takes.

\*Conjugated Estrogens Tablets have been evaluated as probably effective for postmenopausal osteoporosis.

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by physicians who care.**

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BRAND OF  
**CONJUGATED ESTROGENS  
TABLETS, U.S.P.**  
0.3 mg/0.625 mg/1.25 mg/2.5 mg

Please see following page for brief summary of prescribing information.



**BRIEF SUMMARY**  
**(FOR FULL PRESCRIBING INFORMATION,**  
**AND PATIENT INFORMATION, SEE PACKAGE CIRCULAR)**  
**PREMARIN® Brand of Conjugated Estrogens Tablets, U.S.P.**

**1. ESTROGENS HAVE BEEN REPORTED TO INCREASE THE RISK OF ENDOMETRIAL CARCINOMA.**

Three independent case control studies have reported an increased risk of endometrial cancer in postmenopausal women exposed to exogenous estrogens for more than one year.<sup>1-3</sup> This risk was independent of the other known risk factors for endometrial cancer. These studies are further supported by the finding that incidence rates of endometrial cancer have increased sharply since 1969 in eight different areas of the United States with population-based cancer reporting systems, an increase which may be related to the rapidly expanding use of estrogens during the last decade.<sup>4</sup> The three case control studies reported that the risk of endometrial cancer in estrogen users was about 4.5 to 13.9 times greater than in nonusers. The risk appears to depend on both duration of treatment<sup>1</sup> and on estrogen dose.<sup>3</sup> In view of these findings, when estrogens are used for the treatment of menopausal symptoms, the lowest dose that will control symptoms should be utilized and medication should be discontinued as soon as possible. When prolonged treatment is medically indicated, the patient should be reassessed on at least a semiannual basis to determine the need for continued therapy. Although the evidence must be considered preliminary, one study suggests that cyclic administration of low doses of estrogen may carry less risk than continuous administration.<sup>5</sup> If therefore appears prudent to utilize such a regimen. Close clinical surveillance of all women taking estrogens is important. In all cases of undiagnosed persistent or recurring abnormal vaginal bleeding, adequate diagnostic measures should be undertaken to rule out malignancy. There is no evidence at present that "natural" estrogens are more or less hazardous than "synthetic" estrogens at equiestrogenic doses.

**2. ESTROGENS SHOULD NOT BE USED DURING PREGNANCY.**

The use of female sex hormones, both estrogens and progestogens, during early pregnancy may seriously damage the offspring. It has been shown that females exposed in utero to diethylstilbestrol, a non-steroidal estrogen, have an increased risk of developing in later life a form of vaginal or cervical cancer that is ordinarily extremely rare.<sup>6,7</sup> This risk has been estimated as not greater than 4 per 1000 exposures.<sup>8</sup> Furthermore, a high percentage of such exposed women (from 30 to 90 percent) have been found to have vaginal adenosis.<sup>9-12</sup> Epithelial changes of the vagina and cervix have been found to have vaginal adenosis. It is not known whether they are precursors of malignancy. Although similar data are not available with the use of other estrogens, it cannot be presumed they would not induce similar changes. Several reports suggest an association between intrauterine exposure to female sex hormones and congenital anomalies, including congenital heart defects and limb reduction defects.<sup>13-16</sup> One case control study<sup>17</sup> estimated a 4.7-fold increased risk of limb reduction defects in infants exposed in utero to sex hormones (oral contraceptives, hormone withdrawal tests for pregnancy, or attempted treatment for threatened abortion). Some of these exposures were very short and involved only a few days of treatment. The data suggest that the risk of limb reduction defects in exposed fetuses is somewhat less than 1 per 1000. In the past, female sex hormones have been used during pregnancy in an attempt to treat threatened or habitual abortion. There is considerable evidence that estrogens are ineffective for these indications, and there is no evidence from well-controlled studies that progestogens are effective for these uses. If PREMARIN is used during pregnancy or if the patient becomes pregnant while taking this drug, she should be apprised of the potential risks to the fetus, and the advisability of pregnancy continuation.

**DESCRIPTION:** PREMARIN (Conjugated Estrogens Tablets, U.S.P.) for oral administration contains a mixture of estrogens, obtained exclusively from natural sources, blended to represent the average composition of material derived from pregnant mares' urine. It contains estrone, equilin, and 17 $\alpha$ -dihydroequilin, together with smaller amounts of 17 $\alpha$ -estradiol, equilenin, and 17 $\alpha$ -dihydroequilenin as salts of their sulfate esters.

**INDICATIONS:** Based on a review of PREMARIN Tablets by the National Academy of Sciences—National Research Council and/or other information, FDA has classified the indications for use as follows:

**Effective:**

1. Moderate to severe vasomotor symptoms associated with the menopause. (There is no evidence that estrogens are effective for nervous symptoms or depression without associated vasomotor symptoms, and they should not be used to treat such conditions.)
2. Atrophic vaginitis.
3. Kraurosis vulvae.
4. Female hypogonadism.
5. Female castration.
6. Primary ovarian failure.
7. Breast cancer (for palliation only) in appropriately selected women and men with metastatic disease.
8. Prostatic carcinoma—palliative therapy of advanced disease.
9. Postpartum breast engorgement—Although estrogens have been widely used for the prevention of postpartum breast engorgement, controlled studies have demonstrated that the incidence of significant painful engorgement in patients not receiving such hormonal therapy is low and usually responsive to appropriate analgesic or other supportive therapy. Consequently, the benefit to be derived from estrogen therapy for this indication must be carefully weighed against the potential increased risk of puerperal thromboembolism associated with the use of large doses of estrogens.<sup>19</sup>

**PREMARIN HAS NOT BEEN SHOWN TO BE EFFECTIVE FOR ANY PURPOSE DURING PREGNANCY AND ITS USE MAY CAUSE SEVERE HARM TO THE FETUS (SEE BOXED WARNING).**

**"Probably" effective:** For estrogen deficiency-induced osteoporosis, and only when used in conjunction with other important therapeutic measures such as diet, calcium, physiotherapy, and good general health-promoting measures. Final classification of this indication requires further investigation.

**CONTRAINDICATIONS:** Estrogens should not be used in women (or men) with any of the following conditions: 1. Known or suspected cancer of the breast except in appropriately selected patients being treated for metastatic disease. 2. Known or suspected estrogen-dependent neoplasia. 3. Known or suspected pregnancy (See Boxed Warning). 4. Undiagnosed abnormal genital bleeding. 5. Active thrombophlebitis or thromboembolic disorders. 6. A past history of thrombophlebitis, thrombosis, or thromboembolic disorders associated with previous estrogen use (except when used in treatment of breast or prostatic malignancy).

**WARNINGS:** At the present time there is no satisfactory evidence that estrogens given to postmenopausal women increase the risk of cancer of the breast,<sup>17</sup> although a recent study has raised this possibility.<sup>18</sup> There is a need for caution in prescribing estrogens for women with a strong family history of breast cancer or who have breast nodules, fibrocystic disease, or abnormal mammograms. A recent study has reported a 2- to 3-fold increase in the risk of surgically confirmed gallbladder disease in women receiving postmenopausal estrogens.<sup>20</sup>

Adverse effects of oral contraceptives may be expected at the larger doses of estrogen used to treat prostatic or breast cancer or postpartum breast engorgement; it has been shown that there is an increased risk of thrombosis in men receiving estrogens for prostatic cancer and women for postpartum breast engorgement.<sup>19-22</sup> Users of oral contraceptives have an increased risk of diseases, such as thrombophlebitis, pulmonary embolism, stroke, and myocardial infarction.<sup>23-26</sup> Cases of retinal thrombosis, mesenteric thrombosis, and optic neuritis have been reported in oral contraceptive users.<sup>21,27</sup> An increased risk of postsurgery thromboembolic complications has also been reported in users of oral contraceptives.<sup>22,28</sup> If feasible, estrogen should be discontinued at least 4 weeks before surgery of the type associated with an increased risk of thromboembolism, or during periods of prolonged immobilization. Estrogens should not be used in persons with active thrombophlebitis, thromboembolic disorders, or in persons with a history of such disorders in association with estrogen use. They should be used with caution in patients with cerebral vascular or coronary

to treat cancer of the prostate and breast, have been shown<sup>25</sup> to increase the risk of nonfatal myocardial infarction, pulmonary embolism and thrombophlebitis. When doses of this size are used, any of the thromboembolic and thrombotic adverse effects should be considered a clear risk.

Benign hepatic adenomas should be considered in estrogen users having abdominal pain and tenderness, abdominal mass, or hypovolemic shock. Hepatocellular carcinoma has been reported in women taking estrogen-containing oral contraceptives.<sup>29</sup> Increased blood pressure may occur with use of estrogens in the menopause<sup>30</sup> and blood pressure should be monitored with estrogen use. A worsening of glucose tolerance has been observed in patients on estrogen-containing oral contraceptives. For this reason, diabetic patients should be carefully observed. Estrogens may lead to severe hypercalcemia in patients with breast cancer and bone metastases.

**PRECAUTIONS:** Physical examination and a complete medical and family history should be taken prior to the initiation of any estrogen therapy with special reference to blood pressure, breasts, abdomen, and pelvic organs, and should include a Papanicolaou smear. As a general rule, estrogen should not be prescribed for longer than one year without another physical examination being performed. Conditions influenced by fluid retention such as asthma, epilepsy, migraine, and cardiac or renal dysfunction, require careful observation. Certain patients may develop manifestations of excessive estrogenic stimulation, such as abnormal or excessive uterine bleeding, mastodynia, etc. Oral contraceptives appear to be associated with an increased incidence of mental depression.<sup>31,32</sup> Patients with a history of depression should be carefully observed. Preexisting uterine leiomyomata may increase in size during estrogen use. The pathologist should be advised of estrogen therapy when relevant specimens are submitted. If jaundice develops in any patient receiving estrogen, the medication should be discontinued while the cause is investigated. Estrogens should be used with care in patients with impaired liver function, renal insufficiency, metabolic bone diseases associated with hypercalcemia, or in young patients in whom bone growth is not complete.

The following changes may be expected with larger doses of estrogen:

- a. Increased sulfobromophthalein retention.
- b. Increased prothrombin and factors VII, VIII, IX, and X; decreased antithrombin III; increased norepinephrine-induced platelet aggregability.
- c. Increased thyroid binding globulin (TBG) leading to increased circulating total thyroid hormone, as measured by PBI, T<sub>4</sub> by column, or T<sub>4</sub> by radioimmunoassay. Free T<sub>3</sub> resin uptake is decreased, reflecting the elevated TBG; free T<sub>4</sub> concentration is unaltered.
- d. Impaired glucose tolerance.
- e. Decreased pregnanediol excretion.
- f. Reduced response to metyrapone test.
- g. Reduced serum folate concentration.
- h. Increased serum triglyceride and phospholipid concentration.

As a general principle, the administration of any drug to nursing mothers should be done only when clearly necessary since many drugs are excreted in human milk.

**ADVERSE REACTIONS:** The following have been reported with estrogenic therapy, including oral contraceptives: breakthrough bleeding, spotting, change in menstrual flow, dysmenorrhea, premenstrual-like syndrome, amenorrhea during and after treatment, increase in size of uterine fibromyomata, vaginal candidiasis, change in cervical erosion and in degree of cervical secretion, cystitis-like syndrome, tenderness, enlargement, secretion (of breasts), nausea, vomiting, abdominal cramps, bloating, cholestatic jaundice, chloasma or melasma which may persist when drug is discontinued, erythema multiforme, erythema nodosum, hemorrhagic eruption, loss of scalp hair, hirsutism, steepening of corneal curvature, intolerance to contact lenses, headache, migraine, dizziness, mental depression, chorea, increase or decrease in weight, reduced carbohydrate tolerance, aggravation of porphyria, edema, changes in libido.

**ACUTE OVERDOSAGE:** May cause nausea, and withdrawal bleeding may occur in females. **DOSAGE AND ADMINISTRATION:** 1. Given cyclically for short term use only. For treatment of moderate to severe vasomotor symptoms, atrophic vaginitis, or kraurosis vulvae associated with the menopause (0.3 to 1.25 mg or more daily).

The lowest dose that will control symptoms should be chosen and medication should be discontinued as promptly as possible.

Administration should be cyclic (e.g., three weeks on and one week off).

Attempts to discontinue or taper medication should be made at three to six month intervals. 2. Given cyclically: Female hypogonadism. Female castration. Primary ovarian failure. Osteoporosis.

Female hypogonadism—2.5 to 7.5 mg daily, in divided doses for 20 days, followed by a rest period of 10 days' duration. If bleeding does not occur by the end of this period, the same dosage schedule is repeated. The number of courses of estrogen therapy necessary to produce bleeding may vary depending on the responsiveness of the endometrium.

If bleeding occurs before the end of the 10 day period, begin a 20 day estrogen-progestin cyclic regimen with PREMARIN (Conjugated Estrogens Tablets, U.S.P.), 2.5 to 7.5 mg daily in divided doses, for 20 days. During the last five days of estrogen therapy, give an oral progestin. If bleeding occurs before this regimen is concluded, therapy is discontinued and may be resumed on the fifth day of bleeding.

Female castration and primary ovarian failure—1.25 mg daily, cyclically. Adjust upward or downward according to response of the patient. For maintenance, adjust dosage to lowest level that will provide effective control.

Osteoporosis (to retard progression)—1.25 mg daily, cyclically.

3. Given for a few days: Prevention of postpartum breast engorgement—3.75 mg every four hours for five doses, or 1.25 mg every four hours for five days.

4. Given chronically: Inoperable progressing prostatic cancer—1.25 to 2.5 mg three times daily.

Inoperable progressing breast cancer in appropriately selected men and postmenopausal women—10 mg three times daily for a period of at least three months.

Patients with an intact uterus should be monitored for signs of endometrial cancer and appropriate measures taken to rule out malignancy in the event of persistent or recurring abnormal vaginal bleeding.

**HOW SUPPLIED:** PREMARIN (Conjugated Estrogens Tablets, U.S.P.) No. 865—Each purple tablet contains 2.5 mg in bottles of 100 and 1,000. No. 866—Each yellow tablet contains 1.25 mg in bottles of 100 and 1,000. Also in unit dose package of 100. No. 867—Each red tablet contains 0.625 mg in bottles of 100 and 1,000. Also in unit dose package of 100. No. 868—Each green tablet contains 0.3 mg in bottles of 100 and 1,000.

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## COLPOSCOPY

**A Scientific and Practical Approach to the Cervix and Vagina in Health and Disease**

**Second Edition**

By **Malcolm Coppleson**, King George V Memorial Hospital, Sydney, Australia; **Ellis Pixley**, King Edward Memorial Hospital, Perth, Australia; and **Bevan Reid**, Univ. of Sydney, Sydney, Australia. (5 Contributors) This text, rewritten and updated for this Second Edition, continues to offer the gynecologist and obstetrician an unsurpassed account of all aspects of the practice of colposcopy. Scores of new illustrations and photographs have been added to the already profusely illustrated pages of this book.

Emphases of the text remain on colposcopic morphology, histology, instrumentation and nomenclature. Special attention is directed to the transformation zone and to the vaginal epithelium as the seat of diethylstilbestrol (DES) lesions. The authors have used a unique and extensive series of colpophotographs of cases ranging from fetal to postmenopausal ages to establish the life history of the transformation zone.

Correlations of colposcopy and histology — both practical and academic — are made throughout the book, and the section on colposcopic morphology has been amplified to constitute a thorough atlas. The entire range of colposcopic appearances of the original epithelia, typical and atypical transformation zones, overt cancer, infections, condylomata, and miscellaneous conditions are examined.

The authors also provide detailed discussions on the use of colposcopy for the location of lesions in the presence of an abnormal smear report; for the evaluation of the cervical canal; and for the selection of type, site and size of biopsy in the diagnosis of cervical intraepithelial neoplasia (CIN). Special reference is made to conservative measures in the treatment of CIN and microinvasive cancer.

*The Colposcopist* stated in a review of this book, "It is hoped that the publishers will prevail on the authors to publish many more editions of this text so that colposcopists to come will have the benefit of their overview of cervical disease and their keen insight to the newer developments in the field." '78, 512 pp. (6 3/4 x 9 3/4), 306 il. (1 in color), 11 tables, \$40.25

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# American Journal of Obstetrics and Gynecology

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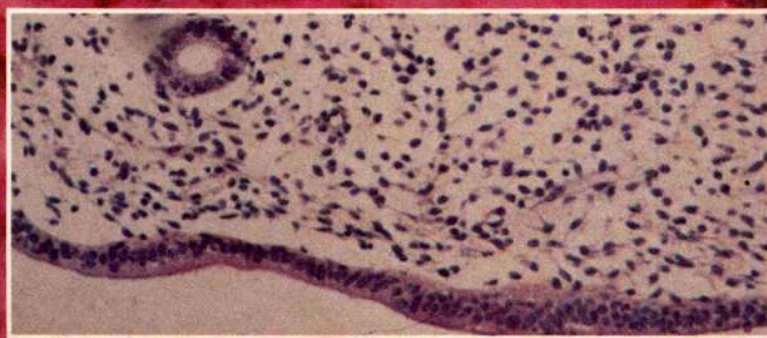
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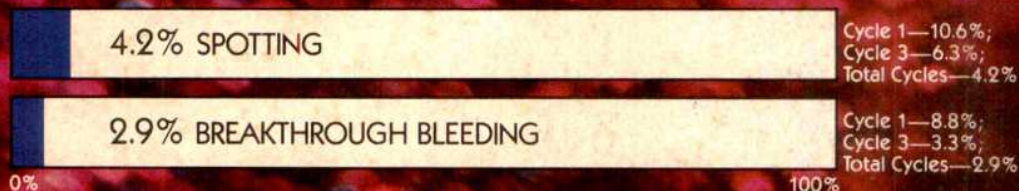


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**Indications and Usage**—LO/OVRAL® is indicated for the prevention of pregnancy in women who elect to use oral contraceptives (OC's) as a method of contraception.

**Contraindications**—OC's should not be used in women with any of the following conditions: 1. Thrombophlebitis or thromboembolic disorders. 2. A past history of deep-vein thrombophlebitis or thromboembolic disorders. 3. Cerebral-vascular or coronary-artery disease. 4. Known or suspected carcinoma of the breast. 5. Known or suspected estrogen-dependent neoplasia. 6. Undiagnosed abnormal genital bleeding. 7. Known or suspected pregnancy (see Warning No. 5). 8. Benign or malignant liver tumor which developed during use of OC's or other estrogen-containing products.

## Warnings

**Cigarette smoking increases the risk of serious cardiovascular side effects from oral contraceptive use. This risk increases with age and with heavy smoking (15 or more cigarettes per day) and is quite marked in women over 35 years of age. Women who use oral contraceptives should be strongly advised not to smoke.**

The use of oral contraceptives is associated with increased risk of several serious conditions, including thromboembolism, stroke, myocardial infarction, hepatic adenoma, gallbladder disease, hypertension. Practitioners prescribing oral contraceptives should be familiar with the following information relating to these risks.

1. **Thromboembolic Disorders and Other Vascular Problems**—An increased risk of thromboembolic and thrombotic disease associated with use of OC's is well established. Three principal studies in Great Britain and 3 in the U.S. have demonstrated increased risk of fatal and nonfatal venous thromboembolism and stroke, both hemorrhagic and thrombotic. These studies estimate that users of OC's are 4 to 11 times more likely than nonusers to develop these diseases without evident cause.

**CEREBROVASCULAR DISORDERS**—In a collaborative American study of cerebrovascular disorders in women with and without predisposing causes, it was estimated that the risk of hemorrhagic stroke was 2.0 times greater in users than nonusers and the risk of thrombotic stroke was 4 to 9.5 times greater in users than in nonusers.

**MYOCARDIAL INFARCTION (MI)**—An increased risk of MI associated with use of OC's has been reported, confirming a previously suspected association. These studies, conducted in the UK, found, as expected, that the greater the number of underlying risk factors for coronary artery disease (cigarette smoking, hypertension, hypercholesterolemia, obesity, diabetes, history of pre-eclampsia toxemia) the higher the risk of developing MI, regardless of whether the patient was an OC user or not. OC's, however, were found to be a clear additional risk factor. In terms of relative risk, it has been estimated that OC users who do not smoke (smoking is considered a major predisposing condition to MI) are about twice as likely to have a fatal MI as nonusers who do not smoke. OC users who are also smokers have about a 5-fold increased risk of fatal MI compared to users who do not smoke, but about a 10- to 12-fold increased risk compared to nonusers who do not smoke. Furthermore, amount of smoking is also an important factor. In determining importance of these relative risks, however, baseline rates for various age groups must be given serious consideration. Importance of other predisposing conditions mentioned above in determining relative and absolute risks has not as yet been quantified, likely like the same synergistic action exists, but perhaps to a lesser extent.

**RISK OF DOSE**—In an analysis of data derived from several national adverse-reaction reporting systems, British investigators concluded that risk of thromboembolism, including coronary thrombosis, is directly related to dose of estrogen in OC's. Preparations containing 100 mcg or more of estrogen were associated with higher risk of thromboembolism than those containing 50-80 mcg. Their analysis did suggest, however, that quantity of estrogen may not be the sole factor involved. This finding has been confirmed in the U.S.

**ESTIMATE OF EXCESS MORTALITY FROM CIRCULATORY DISEASES**—A large prospective study carried out in the UK estimated the mortality rate per 100,000 women per year from diseases of the circulatory system for users and nonusers of OC's according to age, smoking habits, and duration of use. Overall excess death rate annually from circulatory diseases for OC users was estimated to be 20 per 100,000 (ages 15-34—5-100,000; ages 35-44—33/100,000; ages 45-49—140/100,000), risk being concentrated in older women, in those with long duration of use, and in cigarette smokers. It was not possible, however, to examine interrelationships of age, smoking, and duration of use, nor to compare effects of continuous vs. intermittent use. Although the study showed a 10-fold increase in death due to circulatory diseases in users for 5 or more years, all these deaths occurred in women 35 or older. Until larger numbers of women under 35 with continuous use for 5 or more years are available, it is not possible to assess magnitude of relative risk for this younger group. Available data from a variety of sources have been analyzed to estimate risk of death associated with various methods of contraception. Estimates of risk of death for each method combined risk of contraceptive method (e.g., thromboembolic and thrombotic disease in the case of OC's) plus risk attributable to pregnancy or abortion in event of method failure. This latter risk varies with effectiveness of method. The study concluded that mortality associated with all methods of birth control is low and below that associated with childbirth, with the exception of OC's in women over 40 who smoke. Lowest mortality is associated with condom or diaphragm backed up by early abortion. Risk of thromboembolic and thrombotic disease associated with OC's increases with age after about 30 and, for MI, is further increased by hypertension, hypercholesterolemia, obesity, diabetes, or history of pre-eclampsia toxemia, and especially cigarette smoking. Physician and patient should be alert to earliest manifestations of thromboembolic and thrombotic disorders (e.g., thrombophlebitis, pulmonary embolism, cerebrovascular insufficiency, coronary occlusion, retinal thrombosis, and mesenteric thrombosis). Should any of these occur or be suspected, the drug should be discontinued immediately. A 4- to 6-fold increased risk of postsurgery thromboembolic complications has been reported in OC users.

If feasible, OC's should be discontinued at least 4 weeks before surgery of a type associated with increased risk of thromboembolism or prolonged immobilization.

2. **Ocular Lesions**—There have been reports of neuro-ocular lesions such as optic neuritis or retinal thrombosis associated with use of OC's. Discontinue OC's if there is unexplained, sudden or gradual, partial or complete loss of vision; onset of proptosis or diplopia; papilledema; or retinal-vascular lesions, and institute appropriate diagnostic and therapeutic measures.

3. **Carcinoma**—Long-term continuous administration of either natural or synthetic estrogen in certain animal species increases frequency of carcinoma of the breast, cervix, vagina, and liver. Certain synthetic progestogens, none currently contained in OC's, have been noted to increase incidence of mammary nodules, benign

and malignant, in dogs; in humans, a case-control studies have reported an increased risk of endometrial carcinoma associated with prolonged use of exogenous estrogen in postmenopausal women. One publication reported on the first 21 cases submitted by physicians to a registry of cases of adenocarcinoma of the endometrium in women under 40 on OC's. Of cases found in women without predisposing risk factors (e.g., irregular bleeding at the time OC's were first given, polycystic ovaries), nearly all occurred in women who had used a sequential OC. These are no longer marketed. No evidence has been reported suggesting increased risk of endometrial cancer in users of conventional combination or progestogen-only OC's. Several studies have found no increase in breast cancer in women taking OC's or estrogens. One study, however, while also noting no overall increased risk of breast cancer in women on OC's, found an excess risk in subgroups of OC users with documented benign breast disease. Reduced occurrence of benign breast tumors in users of OC's has been well documented. In summary, there is at present no confirmed evidence from human studies of increased risk of cancer associated with OC's. Close clinical surveillance of all women on OC's is, nevertheless, essential. In all cases of undiagnosed persistent or recurrent abnormal vaginal bleeding, appropriate diagnostic measures should be taken to rule out malignancy. Women with a strong family history of breast cancer or with breast nodules, fibrocystic disease, or abnormal mammograms should be monitored with particular care if they elect to use OC's.

4. **Hepatic Tumors**—Benign hepatic adenomas have been found to be associated with use of OC's. One study showed that OC's with high hormonal potency were associated with higher risk than lower potency OC's. Although benign, hepatic adenomas may rupture and may cause death through intra-abdominal hemorrhage. This has been reported in short-term as well as long-term users. Two studies relate risk with duration of use of OC's, the risk being much greater after 4 or more years' use. While hepatic adenoma is rare, it should be considered in women presenting abdominal pain and tenderness, abdominal masses or shock. A few cases of hepatocellular carcinoma have been reported in women on OC's. Relationship of these drugs to this type of malignancy is not known.

5. **Use in or Immediately Preceding Pregnancy, Birth Defects in Offspring, and Malignancy in Female Offspring**—Use of female sex hormones—both estrogenic and progestational agents—during early pregnancy may seriously damage the offspring. It has been shown that females exposed in utero to diethylstilbestrol, a nonsteroidal estrogen, have increased risk of developing in later life a form of vaginal or cervical cancer ordinarily extremely rare. This risk has been estimated to be of the order of 1 in 1,000 exposures or less. Although there is no evidence now that OC's further enhance risk of developing this type of malignancy, such patients should be monitored with particular care if they elect to use OC's. Furthermore, 30 to 90% of such exposed women have been found to have epithelial changes of the vagina and cervix. Although these changes are histologically benign, it is not known whether this condition is a precursor of vaginal malignancy. Male children so exposed may develop abnormalities of the urogenital tract. Although similar data are not available with use of other estrogens, it cannot be presumed they would not induce similar changes. An increased risk of congenital anomalies, including heart defects and limb defects, has been reported with use of sex hormones, including OC's, in pregnancy. One case-control study estimated a 4.7-fold increase in risk of limb-reduction defects in infants exposed in utero to sex hormones (OC's, hormonal withdrawal tests for pregnancy, or attempted treatment for threatened abortion). Some exposures involved only a few days. Data suggest that risk of limb-reduction defects in exposed fetuses is somewhat less than 1 in 1,000 live births. In the past, female sex hormones have been used during pregnancy in an attempt to treat threatened or habitual abortion. There is considerable evidence that estrogens are ineffective for these indications, and there is no evidence from well-controlled studies that progestogens are effective. There is some evidence that triploidy and possibly other types of polyploidy are increased among abortions from women who become pregnant soon after ceasing OC's. Embryos with these anomalies are virtually always aborted spontaneously. Whether there is an overall increase in spontaneous abortion of pregnancies conceived soon after stopping OC's is unknown. It is recommended that, for any patient who has missed 2 consecutive periods, pregnancy should be ruled out before continuing OC's. If the patient has not adhered to the prescribed schedule, the possibility of pregnancy should be considered at time of first missed period, and further use of OC's should be withheld until pregnancy has been ruled out. If pregnancy is confirmed, the patient should be apprised of the potential risks to the fetus, and advisability of continuation of the pregnancy should be discussed. It is also recommended that women who discontinue OC's with intent of becoming pregnant use an alternate form of contraception for a period of time before attempting to conceive. Many clinicians recommend 3 months, although no precise information is available on this to base this. The administration of progestogen-estrogen combinations to induce withdrawal bleeding should not be used as a test of pregnancy.

6. **Gallbladder Disease**—Studies report increased risk of surgically confirmed gallbladder disease in users of OC's and estrogens. In one study, increased risk appeared after 2 years' use and doubled after 4 or 5 years' use. In one of the other studies, increased risk was apparent between 6 and 12 months' use.

7. **Carbohydrate and Lipid Metabolic Effects**—Decrease in glucose tolerance has been observed in a significant percentage of patients on OC's. For this reason, prediabetic and diabetic patients should be carefully observed while on OC's. Increase in triglycerides and total phospholipids has been observed in patients on OC's; clinical significance of this finding remains to be defined.

8. **Elevated Blood Pressure**—Increase in blood pressure has been reported in patients on OC's. In some women, hypertension may occur within a few months of beginning OC's. In the 1st year of use, prevalence of women with hypertension is low in users and may be no higher than that of a comparable group of nonusers. Prevalence in users increases, however, with longer exposure, and in the 5th year of use is 2½ to 3 times the reported prevalence in the 1st year. Age is also strongly correlated with development of hypertension in OC users. Women who previously have had hypertension during pregnancy may be more likely to develop elevation of blood pressure on OC's. Hypertension that develops as a result of taking OC's usually returns to normal after discontinuing the drug.

9. **Headache**—Onset or exacerbation of migraine or development of headache of a new pattern which is recurrent, persistent, or severe, requires discontinuation of OC's and evaluation of the cause.

10. **Bleeding Irregularities**—Breakthrough bleeding, spotting, and amenorrhea are frequent reasons for patients discontinuing OC's. In breakthrough bleeding, as in all cases of irregular

vaginal bleeding, nonhormonal causes should be borne in mind. In undiagnosed persistent or recurrent abnormal bleeding from the vagina, adequate diagnostic measures are indicated to rule out pregnancy or malignancy. If pathology has been excluded, time or change to another OC may solve the problem. Changing to an OC with a higher estrogen content, while potentially useful in minimizing menstrual irregularity, should be done only if necessary, since this may increase risk of thromboembolic disease. Women with past history of oligomenorrhea or secondary amenorrhea or young women without regular cycles may have a tendency to remain anovulatory or to become amenorrheic after discontinuing OC's. Women with these pre-existing problems should be advised of this possibility and encouraged to use other methods. Post-use anovulation, possibly prolonged, may also occur in women without previous irregularities.

11. **Ectopic Pregnancy**—Ectopic as well as intrauterine pregnancy may occur in contraceptive failures.

12. **Breast-feeding**—OC's given in the postpartum period may interfere with lactation and decrease quantity and quality of breast milk. Furthermore, a small fraction of the hormones in OC's has been identified in the milk of mothers on OC's; effects, if any, on the breast-fed child have not been determined. If feasible, defer OC's until infant has been weaned.

**Precautions**—GENERAL—1. A complete medical and family history should be taken prior to initiation of OC's. Pretreatment and periodic physical examinations should include special reference to blood pressure, breasts, abdomen and pelvic organs, including Pap smear and relevant laboratory tests. As a general rule OC's should not be prescribed for longer than 1 year without another physical examination.

2. Under influence of estrogen-progestogen preparations, pre-existing uterine leiomyomata may increase in size.

3. Patients with history of psychic depression should be carefully observed and the drug discontinued if depression recurs to a serious degree. Patients becoming significantly depressed while on OC's should stop OC's and use an alternate method to try to determine whether the symptom is drug-related.

4. OC's may cause some degree of fluid retention. They should be prescribed with caution, and only with careful monitoring, in patients with conditions which might be aggravated by fluid retention, such as convulsive disorders, migraine syndrome, asthma, or cardiac or renal insufficiency.

5. Patients with a past history of jaundice during pregnancy have an increased risk of recurrence while on OC's. If jaundice develops, OC's should be discontinued.

6. Steroid hormones may be poorly metabolized in patients with impaired liver function and should be administered with caution. 7. OC users may have disturbances in normal tryptophan metabolism which may result in a relative pyridoxine deficiency. Clinical significance is undetermined.

8. Serum folate levels may be depressed by OC's. Since the pregnant woman is predisposed to development of folate deficiency and incidence of folate deficiency increases with increasing gestation, it is possible that if a woman becomes pregnant shortly after stopping OC's, she may have a greater chance of developing folate deficiency and complications attributed to this deficiency.

9. The pathologist should be advised of OC therapy when relevant specimens are submitted.

10. Certain endocrine- and liver-function tests and blood components may be affected by estrogen-containing OC's:

a. Increased sulfobromophthalen retention. b. Increased prothrombin and factors VII, VIII, IX, and X, decreased antithrombin 3, increased norepinephrine-induced platelet aggregability. c. Increased thyroid-binding globulin (TBG) leading to increased circulating total-thyroid hormone, as measured by protein-bound iodine (PBI), T4 by column, or T4 by radioimmunoassay. Free T3 resin uptake is decreased, reflecting the elevated TBG. Free T4 concentration is unaltered. d. Decreased pregnandiol excretion. e. Reduced response to metyrapone test.

**Information for the Patient**—See Patient Package Labeling.

**Drug Interactions**—Reduced efficacy and increased incidence of breakthrough bleeding have been associated with concomitant use of rifampin. A similar association has been suggested with barbiturates, phenylbutazone, phenytoin sodium, ampicillin and tetracycline.

**Carcinogenesis**—See Warnings on carcinogenic potential of OC's.

**Pregnancy**—Category X. See Contraindications, Warnings.

**Nursing Mothers**—See Warnings.

**Adverse Reactions**—An increased risk of these serious adverse reactions has been associated with use of OC's (see Warnings): thrombophlebitis, pulmonary embolism, coronary thrombosis, cerebral thrombosis, cerebral hemorrhage, hypertension, gallbladder disease, benign hepatomas, congenital anomalies. There is evidence of an association between the following conditions and use of OC's although additional confirmatory studies are needed: mesenteric thrombosis, neuro-ocular lesions, e.g., retinal thrombosis and optic neuritis.

The following adverse reactions have been reported in patients on OC's and are believed to be drug-related. Nausea and/or vomiting, usually the most common adverse reactions, occur in approximately 10 percent or less of patients during the first cycle. Other reactions, as a general rule, are seen much less frequently or only occasionally. Gastrointestinal symptoms (such as abdominal cramps and bloating); breakthrough bleeding, spotting, change in menstrual flow, dysmenorrhea, amenorrhea during and after treatment, temporary infertility after discontinuance of treatment; edema; chloasma or melasma which may persist; breast changes: tenderness, enlargement, and secretion; change in weight (increase or decrease); change in cervical erosion and cervical secretion; possible diminution in lactation when given immediately postpartum; cholestatic jaundice; migraine; increase in size of uterine leiomyomata; rash (allergic); mental depression; reduced tolerance to carbohydrates; vaginal candidiasis; change in corneal curvature (steepening); intolerance to contact lenses.

The following adverse reactions have been reported in users of OC's, and the association has been neither confirmed nor refuted: premenstrual-like syndrome, cataracts, changes in libido, chorea, changes in appetite, cystitis-like syndrome, headache, nervousness, dizziness, hirsutism, loss of scalp hair, erythema multiforme, erythema nodosum, hemorrhagic eruption, vaginitis, porphyria.

**Acute Overdose**—Serious ill effects have not been reported following acute ingestion of large doses of OC's by young children. Overdose may cause nausea, and withdrawal bleeding may occur in females.

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OBSTETRICS

Pregnancy complicated by maternal heart disease at the National Maternity Hospital, Dublin, Ireland, 1969 to 1978

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Three hundred eighty-seven pregnancies (295 patients) complicated by maternal heart disease, managed at the National Maternity Hospital, Dublin, Ireland, during the years 1969 to 1978, were reviewed. The incidence was 0.5%. Three hundred twenty-three (83.5%) were of rheumatic origin, 52 (13.4%) were congenital, and the remaining 12 (3.1%) were a miscellaneous group and included cases of cor pulmonale and coronary artery disease. There were two maternal deaths—one from congenital heart disease and one from postpartum suicide, unrelated to mild rheumatic heart disease. The perinatal mortality rate was 3.3%. Five pregnancies (three patients) were complicated by surgically uncorrected cyanotic congenital heart disease. One of the maternal deaths and three of the perinatal deaths occurred in this group. There were 38 episodes of cardiac failure (38 patients) in cases of rheumatic heart disease. The New York Heart Association grading was grade 1 in 15 (39%) of these before the onset of failure. Prophylactic antibiotics were not used and infective endocarditis did not occur. Therapeutic abortion was not practiced and a conservative approach was adopted in obstetric intervention and in all drug therapy. (AM. J. OBSTET. GYNECOL. 139:1, 1981.)

THE DECLINE in maternal mortality in countries with well-developed maternity services is directly attributable to the decreased number of deaths resulting from

hemorrhage, infection, and toxemia.<sup>1-3</sup> In contrast, the relative contribution of heart disease to maternal mortality remains unchanged and heart disease is the leading cause of indirect maternal death in Ireland,<sup>1</sup> Great Britain,<sup>2</sup> and parts of the United States.<sup>4</sup> Published estimates of the frequency of heart disease in pregnancy vary from 0.9%<sup>5</sup> to 3.7%.<sup>6</sup> Despite the large collective experience of this problem, many aspects of management remain controversial, e.g., the efficacy of antibiotic prophylaxis of infective endocarditis and the optimum anticoagulant regimen for patients with prosthetic heart valves. The present report emphasizes

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**Table I.** Method of delivery: Comparison of cardiac and noncardiac patients at the National Maternity Hospital, Dublin, Ireland, 1969 to 1978

| Method of delivery   | Cardiac patients |      | All mothers |      |
|----------------------|------------------|------|-------------|------|
|                      | No.              | %    | No.         | %    |
| Spontaneous          | 295              | 76.2 | 56,355      | 75.7 |
| Forceps              | 70               | 18.1 | 6,130       | 8.2  |
| Cesarean section     | 13               | 3.4  | 4,448       | 5.9  |
| Breech               | 2                | 0.5  | 172         | 0.4  |
| Spontaneous abortion | 7                | 1.8  | 6,775       | 9.3  |

particular aspects of patient management which contrast with those practiced elsewhere.

Five case reports of pregnancies complicated by uncorrected cyanotic congenital heart disease are included. They highlight the considerable threat of this condition to maternal and fetal well-being.

**Methods and material**

Patients were seen regularly by a cardiologist and an obstetrician at a combined antenatal-cardiology clinic. The frequency of patients' visits to the clinic varied from weekly to monthly, depending on the cardiac state and duration of gestation. At each visit, a functional grading was made according to the criteria of the New York Heart Association,<sup>7</sup> and, in addition, the severity of the cardiac lesion was assessed on the basis of a detailed clinical examination. Prognostic and therapeutic decisions were based on both criteria, but predominantly on objective signs, as these were found to be more informative. Patients were advised on the importance of adequate rest, of taking iron and folic acid supplements, and of not smoking. Any infection was regarded as serious and was vigorously treated; all patients were screened for asymptomatic urinary tract infection. They were encouraged to report back to hospital without waiting for their next clinical appointment if any deterioration in their condition occurred. Patients with NYHA Classes I and II and those whose signs confirmed that their cardiac lesions were mild were admitted to the hospital routinely at 38 weeks' gestation for bed rest and remained for 2 weeks or longer following delivery. There were occasional patients with grades I and II who were admitted because their signs suggested that heart failure was imminent. Routine antibiotic prophylaxis against endocarditis was not practiced. Patients were encouraged to breast-feed within the obvious limitations imposed by the need for adequate maternal rest and maternal drug therapy. Heart failure was treated with digitalis and diuretics and tachyarrhythmias were treated by drugs alone.

Therapeutic abortion was not performed under any circumstances.

Labor was induced only for the most stringent obstetric indications and likewise cesarean section was performed only where there were clear obstetric indications. During the period under review, a policy of active management of labor was pursued<sup>8,9</sup> and cardiac patients were not excluded. Forceps were not used routinely in Class III and IV patients to expedite the second stage of labor. Perineal infiltration and pudendal nerve block was the usual method of analgesia. Epidural, spinal, and caudal analgesia were not used. Intramuscular ergometrine was given immediately following completion of the second stage, except in patients with heart failure. Sedatives, hypnotics, and tranquilizers were avoided.

**Results**

A total of 77,814 mothers were delivered during the 10-year period. Three hundred eighty-seven pregnancies were complicated by heart disease, an incidence of 0.5%.

In Table I, the method of delivery in cardiac patients is compared with that in noncardiac patients. The major diagnostic categories of heart disease are summarized in Table II. Twenty patients (6.8%) were over the age of 40 and 52 (17.8%) were pregnant for at least the fifth time.

**Rheumatic heart disease.** Rheumatic heart disease complicated 323 pregnancies (245 patients). The distribution of dominant valve defects is shown in Table III. In 88.2% of these pregnancies, patients were Class I or II according to the NYHA. Complications which could be attributed to heart disease occurred in 50 pregnancies: heart failure, 38 (11.8%); paroxysmal supraventricular tachycardia, 2 (0.6%); pulmonary embolism, 2 (0.6%); and atrial fibrillation developing during pregnancy, 7 (2.2%). Four patients had chorea during pregnancy which persisted until after delivery despite treatment with penicillin and phenobarbital and bed rest. The mean age and parity of patients with heart failure did not differ from that in the rheumatic group as a whole. Details of time of onset and type of heart failure, dominant valve lesion, and first-visit NYHA Class are shown in Table IV.

Fifty pregnancies (46 patients) occurred following previous valve surgery. Forty patients had a mitral valvotomy (mean interval between valvotomy and pregnancy, 6.7 years; range, 1 to 16 years), three had prosthetic heart valves inserted (two mitral, one aortic), and three had a mitral valvotomy during pregnancy. The mean valvotomy-pregnancy interval among pa-



Table II. Major diagnostic categories of heart disease, 1969 to 1978

| Type of heart disease | Pregnancies |      | Patients |      | Mean age at diagnosis (yr) | Primigravid patients (%) | Multigravid patients (%) |
|-----------------------|-------------|------|----------|------|----------------------------|--------------------------|--------------------------|
|                       | No.         | %    | No.      | %    |                            |                          |                          |
| Rheumatic             | 323         | 83.5 | 245      | 83.9 | 30.7                       | 24                       | 76                       |
| Congenital            | 52          | 13.4 | 37       | 12.7 | 29.7                       | 20                       | 80                       |
| Miscellaneous         | 12          | 3.1  | 10       | 3.4  | 37.1                       | 0                        | 100                      |
| Total                 | 387         | 100  | 292      | 100  | —                          | —                        | —                        |

tients with NYHA Classes III and IV was 2.8 years longer than among Class I and II patients. Mitral valvotomy was performed three times during pregnancy at 20 (two patients) and 21 weeks. All three patients had tight mitral stenosis and one had an episode of acute pulmonary edema at 17 weeks' gestation. The ensuing pregnancies were uncomplicated and resulted in normal deliveries at term. The three patients with prosthetic valves were maintained on a regimen of oral anticoagulants until the thirty-seventh week. Heparin was then administered intravenously by continuous-infusion pump, adjusted in a dosage which was designed to keep the whole blood clotting time between two and three times normal. The infusion was stopped at the onset of labor and oral anticoagulants were reintroduced 24 hours post partum. Two of the offspring were normal and the third had a birth weight which was less than the fifth centile for gestational age.

**Congenital heart disease.** Fifty-two pregnancies (47 patients) were complicated by congenital heart disease (Table V). One of the offspring had congenital heart disease, an incidence of 1.9%. Pregnancies were unremarkable with the exception of those which were complicated by uncorrected cyanotic congenital heart disease.

**Case reports.** M. R., aged 18, was seen in her first pregnancy. She had a ventricular septal defect, pulmonary hypertension, and right-to-left shunt confirmed at cardiac catheterization at the age of 6. She had clubbing and cyanosis. The hemoglobin was 13 gm/100 ml., i.e., she was probably anemic. She was admitted to the hospital at her first visit, at 27 weeks, and remained at complete bed rest until delivery. Continuous oxygen was administered by nasal catheter at the maximum tolerable flow rate. Spontaneous labor and a normal birth occurred at 39 weeks. The baby weighed 3,070 grams. Central venous pressure was carefully monitored throughout labor with an in-dwelling central venous pressure line. The puerperium was uncomplicated.

K. N., aged 29, was seen in her first pregnancy. Total anomalous pulmonary venous drainage and an atrial septal defect were diagnosed at cardiac catheterization at the age of 15. Pulmonary artery pressure was nor-

Table III. Rheumatic heart disease: Distribution of dominant valve defects

| Dominant valve defect    | Patients |      | Pregnancies |      |
|--------------------------|----------|------|-------------|------|
|                          | No.      | %    | No.         | %    |
| Mitral stenosis:         |          |      |             |      |
| Pure                     | 39       | 16.1 | 43          | 13.5 |
| With mitral incompetence | 78       | 32.2 | 106         | 33.1 |
| Mitral incompetence      | 90       | 37.3 | 122         | 38.1 |
| Aortic regurgitation     | 17       | 7.0  | 25          | 7.8  |
| Aortic stenosis          | 18       | 7.4  | 24          | 7.5  |
| Total                    | 242      | 100  | 320         | 100  |

mal. The patient refused surgery. She had clubbing and cyanosis. The hemoglobin was 18 gm/100 ml. She was attended antenatally from 14 weeks. She was admitted to the hospital with a thrombosis in a left calf vein at 33 weeks and was treated with coumarin. Spontaneous labor occurred at 39 weeks. A live-born infant weighing 1,900 grams (fifth centile for gestational age) was delivered by forceps. The puerperium was uncomplicated.

In this patient's second pregnancy, at age 34, 5,000 U twice daily of subcutaneous calcium heparin was given because of her history. She was attended antenatally from 14 weeks. The hemoglobin was 16.5 gm/100 ml. She was admitted to the hospital for bed rest at 38 weeks. Fetal heart tones could not be heard 6 days later. A macerated stillborn infant weighed 2,420 grams. Cause of death was unknown. The puerperium was uneventful.

A. McM., aged 24, was seen in her first pregnancy. Ventricular septal defect, pulmonary hypertension, and right-to-left shunt were diagnosed at cardiac catheterization at age 11 years. She had clubbing and cyanosis. The hemoglobin was 20 gm/100 ml. She was attended antenatally from 9 weeks. The patient remained at bed rest at home (her husband was a doctor) until admission at 27 weeks for complete bed rest in the hospital. A lower-segment cesarean section was performed at 35 weeks because of fetal dysmaturity. A central venous pressure line was maintained throughout operation, with blood transfusion to maintain central venous pressure at 10 to 15 mm Hg. The baby weighed 620 grams and died at 48 hours of age. Postmortem diagnosis was pulmonary hemorrhage.



**Table IV.** Heart failure in 38 pregnancies complicated by rheumatic heart disease

| Type of failure       | No. of patients | Dominant valve lesion |    |    |    | Time of onset |        |       |            | NYHA Class before the onset of failure |    |     |    |
|-----------------------|-----------------|-----------------------|----|----|----|---------------|--------|-------|------------|----------------------------------------|----|-----|----|
|                       |                 | MS                    | MI | AS | AI | Trimester     |        |       | Puerperium | U                                      | II | III | IV |
|                       |                 |                       |    |    |    | First         | Second | Third |            |                                        |    |     |    |
| Pulmonary congestion  | 20              | 7                     | 10 | 2  | 1  | 1             | 4      | 15    | 0          | 9                                      | 8  | 0   | 3  |
| Acute pulmonary edema | 9               | 4                     | 3  | 2  | 2  | 1             | 0      | 3     | 5          | 5                                      | 3  | 0   | 1  |
| Congestive failure    | 9               | 3                     | 5  | 2  | 0  | 1             | 0      | 6     | 2          | 1                                      | 4  | 2   | 2  |

MS: Mitral stenosis; MI: mitral incompetence; AS: aortic stenosis; AI: aortic incompetence.

**Table V.** Type of congenital heart disease complicating 52 pregnancies (47 patients)

| Heart lesion                                       | Patients |      | Pregnancies |      | No. of pregnancies in patients with uncorrected defect |
|----------------------------------------------------|----------|------|-------------|------|--------------------------------------------------------|
|                                                    | No.      | %    | No.         | %    |                                                        |
| Atrial septal defect                               | 18       | 38.2 | 14          | 26.9 | 4                                                      |
| Ventricular septal defect                          | 6        | 12.9 | 8           | 15.4 | 7                                                      |
| Tetralogy of Fallot                                | 6        | 12.8 | 7           | 13.5 | 0                                                      |
| Coarctation of the aorta                           | 3        | 6.4  | 5           | 9.7  | 1                                                      |
| Pulmonary stenosis                                 | 3        | 6.4  | 4           | 7.6  | 4                                                      |
| Aortic stenosis                                    | 4        | 8.6  | 4           | 7.6  | 3                                                      |
| Patent ductus arteriosus                           | 2        | 4.2  | 3           | 5.8  | 1                                                      |
| Eisenmenger complex                                | 2        | 4.2  | 3           | 5.8  | 3                                                      |
| Total anomalous                                    |          |      |             |      |                                                        |
| Pulmonary venous drainage and atrial septal defect | 1        | 2.1  | 2           | 3.9  | 2                                                      |
| Congenital heart block                             | 1        | 2.1  | 1           | 1.9  | 1                                                      |
| Dextrocardia and aortic stenosis                   | 1        | 2.1  | 1           | 1.9  | 1                                                      |

**Table VI.** Cause of fetal death and the associated maternal heart lesion

|                 | Cause of death                   | No. of deaths | Maternal heart disease                                            |
|-----------------|----------------------------------|---------------|-------------------------------------------------------------------|
| Stillbirths     | Pulmonary hemorrhage             | 1             | Eisenmenger complex                                               |
|                 | Intrauterine anoxia              | 6             | Classes I, IV, and IV RHD: TAPVD and ASD; ASD and angina pectoris |
|                 | Prolapsed cord                   | 1             | Class I RHD                                                       |
|                 | Abruptio placentae               | 1             | Class I RHD                                                       |
|                 | Hemolytic disease of the newborn | 1             | Class I RHD                                                       |
|                 | Congenital heart disease         | 3             | ASD; Classes I and II RHD                                         |
| Neonatal deaths |                                  |               |                                                                   |

RHD: Rheumatic heart disease; TAPVD: total anomalous pulmonary venous drainage; ASD: atrial septal defect.

Apart from a syncopal attack on the sixth day post partum, the puerperium was uncomplicated.

In her second pregnancy, at aged 27, she was more breathless than during the previous pregnancy, but she had no nocturnal dyspnea. The hemoglobin was 21.4 gm/100 ml. She was attended antenatally from 18 weeks. She remained at bed rest at home until admission to hospital for rest at 27 weeks. Continuous oxygen was administered at the maximum tolerable flow rate. She had mild pre-eclampsia at 30 weeks. Lower-segment cesarean section was performed at 33 weeks because of fetal dysmaturity. The baby weighed 1,045 grams (fifth centile for gestational age) and continues well. Sudden maternal death occurred on the ninth day post partum. Postmortem examination revealed histologic evidence of pulmonary hypertension, with a defect 1.5 cm in diameter in the membranous portion of

the ventricular septum. No immediate cause of death was found.

**Fetal deaths.** The total number of infants in the series was 393, of whom 373 (94.9%) survived. There were 10 (1.5%) stillbirths, three (0.8%) neonatal deaths and seven (1.8%) spontaneous abortions. The causes of death, based on postmortem findings and the associated maternal heart disease, are shown in Table VI.

The other maternal death occurred in a 26-year-old primigravid patient with mild aortic stenosis (NYHA Class I). Spontaneous delivery of a healthy living infant took place at 34 weeks. On the fifth postnatal day the patient died following a fall from a second-floor hospital window. There was no history of mental illness.



### Comment

The majority of pregnancies complicated by heart disease are uneventful, with a favorable outcome for both mother and fetus. Adequate daily rest and intense treatment of anemia and infection are essential. The 3.3% perinatal loss in the present series reflects both the remarkably good fetal prognosis in pregnancy complicated by heart disease and the decline in perinatal mortality rates in general. Rates of perinatal loss in previously reported series have varied from 5.7%<sup>10</sup> to 5.2%.<sup>11</sup>

The single maternal death directly attributable to heart disease in this report is in marked contrast to the large number of maternal deaths caused by heart disease in maternal mortality reports. Heart disease accounted for 10.4%, 8.5%, and 12.3%, respectively, of maternal deaths in England and Wales<sup>2</sup> from 1952 to 1972, in Ireland<sup>1</sup> from 1964 to 1973, and in California<sup>4</sup> from 1960 to 1977. Cardiac failure is the most common cause of death.<sup>1, 2, 4</sup>

It has been customary to emphasize the time of maximum occurrence of cardiac failure in pregnancy. We would emphasize that failure can occur at any time during pregnancy or the puerperium and constant vigilance for premonitory signs is required if mortality from this cause is to be reduced. Moreover, sudden unpredictable changes occur in NYHA Classes I to IV and a good functional classification should not engender any relaxation in this vigilance.

The numerically small group with cyanotic disease is disproportionately important in terms of cardiologic and obstetric complications. The Eisenmenger syndrome is associated with a particularly high maternal mortality. There were 12 maternal deaths (18.7%) in 104 pregnancies (64 patients) complicated by this syndrome and reported in the literature.<sup>12-16</sup> Moreover, it was the specific lesion involved in 16 of 44 maternal deaths due to congenital heart disease in England and Wales, from 1961 to 1972.<sup>2</sup> Postpartum syncope and sudden death are typical of the condition. Death is usually attributed either to a rapid increase in the pulmonary vascular resistance or to a decrease in the systemic vascular resistance, with a concomitant increase in right-to-left shunt. The cause of these hemodynamic changes is often unknown, but it is of interest that pre-eclampsia, which was present antenatally in the Eisenmenger-related death in this series, is associated with a sudden fall in systemic vascular resistance soon after delivery.<sup>17</sup> Hemodynamic monitoring has shown sudden changes in pulmonary and right ventricular pressures with posture and straining, and these may precipitate fatal arrhythmias.<sup>18</sup> Immediate replacement

of blood loss,<sup>19</sup> bed rest for symptomatic patients,<sup>14</sup> and trial of supplemental oxygen<sup>14</sup> are the only specific therapeutic measures which are of value.

Our experience of patients with previous mitral valvotomy demonstrates that such surgery does not guarantee immunity from cardiovascular complication in subsequent pregnancies. The longer the valvotomy-pregnancy interval in patients with NYHA Classes III and IV compared to patients with Classes I and II suggests that, in general, families should be completed with the minimum of delay following valvotomy.

Previous reports suggest that pregnancy is not an unacceptable hazard in patients with prosthetic valves.<sup>20</sup> Patients given a prosthetic valve are generally believed to require lifelong anticoagulants to reduce the risk of thromboembolic complications.<sup>21</sup> The optimum anticoagulant regimen for these patients during pregnancy is controversial. The teratogenic potential of the coumarin derivatives is well established.<sup>22-27</sup> A recent review of the maternal and fetal sequelae of anticoagulation during pregnancy has shown that the teratogenicity of the coumarins is independent of the timing of their administration in relation to organogenesis.<sup>28</sup> Moreover, the use of heparin, the primary alternative to the coumarins, did not result in a significantly better outcome of pregnancy because of an increased incidence of stillbirths and premature delivery. The small number of patients in the present series with prosthetic valves precludes a definitive conclusion concerning the safety and efficacy of our anticoagulant regimen. Alternative anticoagulant regimens, including subcutaneous heparin<sup>27</sup> and the use of antiplatelet drugs, have been suggested, but further work is required to establish their efficacy.

Antibiotic prophylaxis of infective endocarditis at delivery and in the puerperium has become an established practice in many centers. The high mortality of infective endocarditis certainly calls for every preventive measure in patients at risk. Justification for using antibiotics is based on the occurrence of transient asymptomatic bacteremia in none to 5% of mothers<sup>29-32</sup> at delivery and on reports of endocarditis in the puerperium.<sup>33-36</sup> However, there is no evidence that the long-established antibiotic regimens have an effect on the frequency with which endocarditis occurs following high-risk procedures. In two recent series totaling over 3,915 women with rheumatic or congenital heart disease<sup>37, 38</sup> in whom routine peripartum antibiotics were not used, there were only two cases (0.05%) of infective endocarditis, both of which were equivocally related to preceding childbirth. Moreover, routine antibiotic prophylaxis carries the dual risks of drug toxicity and



antibiotic-resistant endocarditis.<sup>39</sup> A critical reappraisal of antibiotic prophylaxis in cardiac patients around the time of delivery is required.

Professor Kieran O'Driscoll and Dr. Declan Meagher during their Masterships were responsible for the care of many of the cases.

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# The nonstress test: A review of 3,000 tests

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For the antepartum evaluation of fetal well-being in the high-risk pregnancy, the nonstress test (NST) has been found to be clinically efficacious. The basis for its interpretation has been the presence (reactive or normal) or absence (nonreactive or abnormal) of fetal heart rate accelerations in response to fetal activity. If a nonreactive NST was encountered, the fetus was evaluated further with the contraction stress test (CST). Between July 5, 1977, and October 20, 1979, 3,000 NSTs were performed on 1,452 women with high-risk pregnancies. The NST interpretations were reactive (85.4%); nonreactive (14.0%), and unsatisfactory (0.6%). While the reactive group experienced a favorable fetal outcome, the nonreactive group demonstrated a significant increase in the overall cesarean section rate, the rate of cesarean sections for fetal distress, and the perinatal mortality rate. Based on our experience, the NST continues to be a valuable procedure for the assessment of fetal well-being in our high-risk pregnancies. (AM. J. OBSTET. GYNECOL. 139:7, 1981.)

THE ANTEPARTUM ASSESSMENT of fetal well-being has become an integral part of the management of any high-risk pregnancy. In order to achieve this evaluation, various biochemical and biophysical techniques have been described. The biophysical techniques, primarily antepartum fetal heart rate testing (AFHRT), have enabled the clinician to immediately and reliably evaluate fetal status. Initially, the oxytocin challenge test or currently the contraction stress test (CST) served this function.<sup>1</sup> Even though the time required to perform a CST represented only a fractional part of that period of fetal life, the CST, when negative, has accurately predicted fetal well-being.<sup>2</sup> Despite its proved clinical efficacy, the CST has had a limited application clinically. For example, pregnancies complicated by placenta previa, premature rupture of the membranes, or premature labor have been considered contrain-

dications to a CST. In addition to these clinical shortcomings, the CST is time consuming and requires several hours to complete.

During this time, Lee and associates<sup>3</sup> demonstrated that intrapartum fetal heart rate (FHR) accelerations were a sign of fetal well-being. This factor was later incorporated into a new antepartum testing technique, the fetal activity-acceleration determination (FAD)<sup>4</sup> or presently the nonstress test (NST). This method, which relies on the FHR response to fetal movement rather than to contractions, can be applied to a wider range of clinical conditions. Furthermore, instead of a weekly CST on each high-risk pregnancy, a CST was only necessary in those circumstances where a fetus failed to exhibit the prescribed quantity of FHR accelerations in response to fetal activity or had a nonreactive test. A fetus with a reactive test did not need a CST. Other investigations that involved looking at the relationship between FHR accelerations and the oxytocin challenge test<sup>5</sup> or at perinatal mortality rates in the NST compared to the CST<sup>6,7</sup> have also substantiated the predictive value of nonstress testing. In a prospective study done at our institution which compared the FAD with the oxytocin challenge test, the NST was also shown to be a reliable predictor of outcome.<sup>8</sup>

Since the initial work of Lee and associates,<sup>4</sup> additional prospective NST investigations, including a follow-up study by Lee and Drukker,<sup>9</sup> have been published which dealt primarily with high-risk pregnancies.<sup>10-12</sup> In addition, the NST has been shown to be of value as an outpatient approach<sup>13</sup> as well as in the low-risk pregnancy.<sup>14</sup>

The purpose of the present investigation is to report

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**Table I.** Indications for AFHRT

| Indication                                         | No. | %    |
|----------------------------------------------------|-----|------|
| Postdates                                          | 612 | 35.3 |
| Suspected IUGR                                     | 359 | 20.7 |
| Diabetes mellitus:                                 | 146 | 8.4  |
| Class A                                            | 122 | —    |
| Classes B to R                                     | 24  | —    |
| Pre-eclampsia                                      | 133 | 7.7  |
| Decreased fetal activity                           | 122 | 7.0  |
| Chronic hypertension                               | 98  | 5.6  |
| Previous fetal death                               | 51  | 2.9  |
| Cardiovascular disease<br>(excluding hypertension) | 24  | 1.3  |
| Renal disease                                      | 21  | 1.3  |
| Pulmonary disease                                  | 20  | 1.2  |
| Third-trimester bleeding                           | 16  | 0.9  |
| Endocrine disorders                                | 15  | 0.8  |
| Multiple gestation                                 | 14  | 0.8  |
| Polyhydramnios                                     | 12  | 0.7  |
| Anemia                                             | 12  | 0.7  |
| Rh disease                                         | 7   | 0.4  |
| Other                                              | 74  | 4.3  |

**Table II.** NST results (N = 3,000)

| Result               | No.   | %    |
|----------------------|-------|------|
| Reactive:            | 2,563 | 85.4 |
| No fetal stimulation | 1,921 | 64.0 |
| Fetal stimulation    | 642   | 21.4 |
| Nonreactive          | 419   | 14.0 |
| Unsatisfactory       | 18    | 0.6  |

our experience with the use of the NST as the primary antepartum test for the assessment of fetal well-being in our high-risk pregnancies.

### Materials and methods

All patients whose pregnancies were categorized at high risk for uteroplacental insufficiency were included in the study. Primary indications for antepartum evaluation by the NST have included prolonged pregnancy ( $\geq 42$  weeks), clinically suspected intrauterine growth retardation (IUGR), chronic hypertension, pre-eclampsia, diabetes mellitus, previous fetal death, or decreased fetal activity. Corometrics Types 101B, 111, and 110AC fetal monitors were used to continuously record the FHR and fetal activity. During each NST or CST, the patient was in bed in the semi-Fowler's position. Whenever she felt fetal movement (FM), she was requested to push the 50 mm Hg button on the monitor or the remote button. If four or more FHR accelerations of at least 15 bpm in response to four or more FM were observed within 20 minutes, a reactive test (RNFS) was recorded. Should the fetus fail to react within 20 minutes, he was stimulated by shaking and monitored for an additional 20 minutes. After

fetal stimulation, the FHR and activity were recorded for an additional 20 minutes. If an adequate FHR response was obtained, a reactive test (RFS) was recorded. Whenever a reactive test (RNFS or RFS) was obtained, a CST was not done. If the fetus remained unresponsive after stimulation, the NST was interpreted as nonreactive (NR). Those patients who exhibited a NR NST and later those who exhibited a cord pattern<sup>15</sup> were studied further by the CST. The latter group was included because of the increased risk of fetal death associated with a cord pattern.<sup>15</sup> The procedure for the performance of CST has been previously described.<sup>1</sup> The interpretation of each CST was based on the presence or absence of late deceleration of the FHR in association with at least three uterine contractions over a 10-minute interval. Following a negative CST, a NST was done initially the next week. If a suspicious or positive CST was exhibited, subsequent management was based on fetal maturity. In the presence of an immature fetus, a positive CST was repeated within 24 hours. On the other hand, any patient who exhibited a positive CST and had a mature fetus was delivered expeditiously.<sup>16</sup>

### Results

Between July 5, 1977, and October 20, 1979, 1,452 pregnant women with high-risk pregnancies underwent 3,000 NSTs. The most common indications for fetal evaluation were postdates and clinically suspected IUGR (Table I). Several women had more than one indication for testing.

As illustrated by Table II, the majority of the NSTs (85.4%) were interpreted as reactive. Of the 2,563 reactive tests, 78.8% did not require fetal stimulation and 21.2% did. Overall, 14% of the 3,000 NSTs were considered nonreactive.

CSTs were done for a variety of indications, which included a NR NST, a reactive NST with a cord pattern, a baseline FHR tachycardia (FHR  $\geq 160$  bpm), or an uninterpretable NST (Table III). Of the 470 CSTs done, the majority were considered negative (74.3%). In those instances where a suspicious CST was obtained, the patients were either delivered or had a repeat CST.

A positive CST which followed either a NR NST or a suspicious CST was encountered on 31 occasions (6.7%). Of these 31 patients, 21 were allowed to labor and five (23.8%) required cesarean section for persistent late decelerations. These results are consistent with those in a larger series of patients reported from our institution.<sup>16</sup>

FHR decelerations were observed on 170 NSTs of 151 high-risk pregnancies (10.4%). These NSTs were



Table III. Primary CST results for the entire NST population by indication for CST

| CST results    | NST nonreactive | NST unsatisfactory | NST reactive-CP* | Other† | Total |
|----------------|-----------------|--------------------|------------------|--------|-------|
| Negative       | 307             | 6                  | 34               | 2      | 349   |
| Suspicious     | 76              | 1                  | 12               | 2      | 91    |
| Positive       | 27              | 0                  | 1                | 0      | 28    |
| Not done       | 9               | 7                  | 65               | 0      | 81    |
| Unsatisfactory | 0               | 2                  | 0                | 0      | 2     |
| Repeat NST     | 0               | 2                  | 0                | 0      | 2     |

\*Cord pattern.  
†Baseline FHR tachycardia.

Table IV. Spontaneous CST related to NST results

| CST results | Reactive NST | Nonreactive NST |
|-------------|--------------|-----------------|
| Negative    | 219          | 47              |
| Suspicious  | 6            | 8               |
| Positive    | 3            | 5*              |

\* $\chi^2 = 9.2$ ;  $p < 0.01$ .

interpreted as either reactive (112) or nonreactive (58). After completing a review of this pattern, we had recommended a CST whenever this pattern was observed during a NST.<sup>15</sup> As a result, many of the patients did not have a CST done initially (Table III). As before, many of these patients had suspicious CSTs. Of note, 53.6% of these pregnancies were noted to have an abnormal cord position at delivery. These results are consistent with our previous data.<sup>15</sup>

Spontaneous CSTs were observed during reactive and NR NSTs (9.9%). The results are depicted in Table IV. The incidence of positive CSTs that were observed in the reactive (1.2%) and nonreactive (8.3%) groups was similar to that of a previous investigation done at our institution.<sup>8</sup> In the present investigation, the incidence of spontaneous positive CSTs in the NR group was significantly higher ( $\chi^2 = 9.2$ ,  $p < 0.01$ ). In the three pregnancies which exhibited a reactive NST and a positive CST, two were delivered vaginally and one underwent cesarean section for fetal distress.

The pregnancy outcomes for those women who were delivered within 7 days of their last NST are shown in Table V. When compared with the reactive group, the nonreactive group had a significant increase in cesarean sections, cesarean sections for fetal distress (persistent late decelerations, severe variable decelerations, or sustained FHR bradycardia), meconium-stained amniotic fluid, and perinatal deaths. Apgar scores were similar for both groups. The neonatal deaths are listed in Table VI.

Comment

In this large series of high-risk pregnancies, the NST, when reactive, does represent a satisfactory indi-

Table V. Pregnancy outcome based on delivery within 7 days of last NST by NST results

| Item                    | Reactive NST |      | Nonreactive NST |      |
|-------------------------|--------------|------|-----------------|------|
|                         | No.          | %    | No.             | %    |
| No.                     | 1,001        |      | 235             |      |
| Mode of delivery:       |              |      |                 |      |
| Vaginal                 | 819          | 81.9 | 176             | 74.7 |
| Cesarean section        | 182          | 18.1 | 59*             | 25.3 |
| Fetal distress          | 29           | 2.9  | 20†             | 8.5  |
| Meconium                | 160          | 16.0 | 57‡             | 24.3 |
| Abnormal cord positions | 255          | 25.4 | 61              | 26.0 |
| Perinatal deaths:       |              |      |                 |      |
| Intrauterine            | 3            | —    | 2               | —    |
| Neonatal                | 1            | —    | 5               | —    |
| Rate/1,000              | 4            | —    | 29.4§           | —    |

\* $\chi^2 = 5.8$ ;  $p < 0.05$ .  
† $\chi^2 = 15.8$ ;  $p < 0.01$ .  
‡ $\chi^2 = 9.0$ ;  $p < 0.01$ .  
§ $\chi^2 = 14.4$ ;  $p < 0.01$ .

Table VI. Neonatal deaths observed in the NST population by NST results

| Case No. | NST result  | Cause of death                  |
|----------|-------------|---------------------------------|
| 1        | Reactive    | Infection—Group B streptococcus |
| 2        | Nonreactive | Necrotizing enterocolitis       |
| 3        | Nonreactive | Potter's syndrome               |
| 4        | Nonreactive | Hirschsprung's disease          |
| 5        | Nonreactive | Diaphragmatic hernia            |
| 6        | Nonreactive | Sudden infant death syndrome    |

cator of fetal well-being. This fact is substantiated by the significantly lower incidence of operative deliveries, meconium-stained amniotic fluid and perinatal deaths. The higher incidence of spontaneous negative CSTs, a sign of fetal well-being,<sup>2</sup> observed during a reactive NST provides additional supportive evidence. The fact that three false negative NSTs were observed should not detract from its proved reliability.<sup>4, 6-14</sup>

Nonreactive NSTs which required CSTs were observed more frequently in our investigation than in others.<sup>9, 12-14</sup> While maternal cigarette smoking,<sup>17</sup> fetal somnolence,<sup>4</sup> or prematurity may diminish fetal reac-



tivity, different approaches to the NR NST, like repeating the NST after food intake or stopping the CST when FHR accelerations are observed, appear to be the major differences. On the whole, a NR NST does signify an ominous fate for the fetus.

In general, severe fetal jeopardy is commonly associated with a positive CST.<sup>12</sup> A similar finding was also observed in our NR NST-positive CST group. Of interest, reactive NSTs with a positive CST may not be as ominous. However, the presence of a positive CST in

any NST should not be disregarded. For example, in our series, one patient (33%) did require cesarean section for fetal distress. This is in marked contrast with the results of Schiffrin and associates,<sup>14</sup> where four patients had reactive NSTs-positive CSTs and were delivered without incident.

In conclusion, the NST, when supplemented by the CST for fetal nonreactivity, does represent a clinically reliable technique for the antepartum assessment of fetal well-being.

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# Pregnancy outcome in the patient with a nonreactive nonstress test and a positive contraction stress test

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The pregnancy outcomes for 41 patients with high-risk pregnancies who had undergone antepartum fetal heart rate testing between July 5, 1977, and April 10, 1980, and demonstrated a nonreactive nonstress test (NST) and a positive contraction stress test (CST) were reviewed. The estimated gestational ages of the time of the positive CST ranged from 28 to 44 weeks' gestation. Of the 41 pregnant women with a positive CST, 30 were allowed to labor. The remaining 11 patients underwent immediate cesarean section (8) or were managed expectantly because of immature phospholipid profiles (3). In the laboring group, 60.0% had evidence of late decelerations. Cesarean section for persistent late decelerations was necessary in 33.3%. The overall cesarean section rate for the positive CST group was 58.5%. *The neonatal outcome for these 41 pregnancies demonstrated 29.3% of fetuses to be growth retarded and 17.1% of fetuses to have congenital abnormalities. The overall perinatal mortality rate was 7.3%. This investigation further substantiates the increased fetal and neonatal risks associated with a nonreactive NST and a positive CST. Induction of labor with direct fetal monitoring should be attempted in a term gestation where the cervix is ready for induction of labor and where rapid intervention by cesarean section is possible.* (AM. J. OBSTET. GYNECOL. 139:11, 1981.)

ANTEPARTUM FETAL HEART RATE (FHR) testing has been shown to be a valuable adjunct in the management of the high-risk pregnancy.<sup>1, 2</sup> The goal of this biophysical technique is to identify those fetuses at risk for uteroplacental insufficiency. Currently, the nonstress test (NST) has been used as the primary approach for assessing fetal well-being. When primarily the NST is used, a nonreactive (NR) NST has been

associated with a higher perinatal mortality rate and intrapartum fetal distress than a reactive NST.<sup>2</sup> However, further evaluation with the contraction stress test (CST) is required to improve its predictive accuracy. Whenever the CST is used, the positive CST, which is manifested as repetitive late decelerations of the FHR, carries the gravest prognosis for the fetus. Previous investigations of the pregnancy outcome in patients with a positive CST along have shown higher incidences of intrapartum fetal distress and perinatal death.<sup>3, 4</sup> Braly and Freeman<sup>5</sup> have shown an even poorer outcome in those pregnancies with a positive CST that is not reactive. A recent review by Gauthier and associates<sup>6</sup> of their experience with patients who exhibited a NR NST and a positive CST has shown a similar relationship.

Therefore, an investigation was undertaken at our institution to review and report the clinical outcome and course of those pregnancies in our NST population who had a NR NST and a positive CST.

## Material and methods

Since July 5, 1977, all pregnancies at high-risk for uteroplacental insufficiency have been evaluated by antepartum FHR testing. The indications for antepartum evaluation by the NST were postdates ( $\geq 42$  weeks' ges-

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**Table I.** Clinical profile of those pregnancies which exhibited a NRNST-positive CST

| Item                                                      | Mean value | Range |
|-----------------------------------------------------------|------------|-------|
| Age (years)                                               | 24.3       | 16-39 |
| Gravidity                                                 | 2.0        | 1-6   |
| Parity                                                    | 0.6        | 0-3   |
| Estimated gestational age at time of positive CST (weeks) | 39.0       | 28-44 |

**Table II.** Indications for antepartum FHR testing in those pregnancies with a NRNST-positive CST

| Indication                         | No. | %    |
|------------------------------------|-----|------|
| Postdates                          | 16  | 33.3 |
| Suspected IUGR                     | 14  | 29.2 |
| Pre-eclampsia                      | 6   | 12.5 |
| Chronic hypertension               | 4   | 8.3  |
| Previous fetal death               | 4   | 8.3  |
| Diabetes mellitus (Classes B to D) | 3   | 6.3  |
| Anemia                             | 1   | 2.1  |

tation), clinically suspected intrauterine growth retardation (IUGR), chronic hypertension, pre-eclampsia, diabetes mellitus, previous intrauterine fetal death (IUFD), or decreased fetal movement. Corometrics Types 101B, 111, 110AC, and 112 fetal monitors were used to continuously record the FHR and fetal activity. During each NST and CST, each pregnant woman was in bed in the semi-Fowler's position and blood pressure measurements were taken at 10-minute intervals.

The interpretation of the NST, which depends on the presence of FHR accelerations in response to fetal activity, for our institution has been previously described.<sup>7</sup> Whenever a NR NST was obtained, a CST was done the same day. The CSTs were performed and interpreted according to the method described by Ray and associates.<sup>8</sup>

Whenever a positive CST was obtained, subsequent management was based on fetal maturity and whether the cervix was suitable for induction of labor. If a fetus was considered clinically mature, delivery was accomplished in the most expeditious manner. For example, if the cervix was not suitable for induction of labor or direct FHR monitoring could not be applied, a primary cesarean section was done immediately. On the other hand, if the cervix was suitable for induction of labor, following amniotomy and application of direct FHR monitoring to the fetal vertex, labor was induced with the patient in the left lateral recumbent position.

In cases where fetal maturity was questionable, amniocentesis was performed. If pulmonary maturity was demonstrated by amniotic fluid phospholipid assay (lecithin/sphingomyelin [L/S] ratio  $\geq$  2:1, phosphoti-

dylglycerol present), then the patient was delivered as above. However, if the amniotic fluid indicated pulmonary immaturity, the CST was repeated within 24 hours. If the CST was again positive, subsequent management was based on estriol determinations, especially low or falling values, and joint consultation with the neonatologists.

Finally, all the maternal and neonatal records of those patients with a NR NST and a positive CST were analyzed.

**Results**

Between July 5, 1977, and April 10, 1980, 1,767 women with high-risk pregnancies underwent 3,621 NSTs and 41 were found to have a NR NST and a positive CST (Table I). A total of 44 positive CSTs were recorded. Seventeen patients (41.5%) were cigarette smokers.

The indications for antepartum FHR testing are listed in Table II. Several patients had more than one indication for evaluation. With exception of the pre-eclamptic group, the NST indications for this group were similar to those of our overall NST population.

Four patients had positive CSTs between 28 and 36 weeks' gestation and demonstrated immature amniotic fluid phospholipid profiles (Table III). (The pregnancy outcome for one patient who had positive CSTs at 28 and 36 weeks' gestation is included in the group with no fetal distress in Table III.) A repeat CST was done within 24 hours in each case, and the interpretations were negative (2), suspicious (1), and positive (1). In the pregnancy with two consecutive positive CSTs at 34 weeks' gestation, in a patient with Class D diabetes, IUFD occurred less than 24 hours after the last CST. Technical difficulties precluded us from obtaining the estriol results prior to fetal death. In retrospect, the results were indicative of severe fetal jeopardy.

At delivery, the baby had a tight nuchal cord and appeared normal. At autopsy, the baby was found to be a 34-week infant who was appropriate for gestational age, and no apparent cause of the fetal death was identified. In the patient whose second CST was suspicious at 28 weeks' gestation, subsequent NSTs were reactive. At 36 weeks' gestation, she once again exhibited a NR NST and a positive CST. At this time, she underwent induction of labor. There was no intrapartum evidence of fetal distress, and she was delivered vaginally of a growth-retarded infant with a 5-minute Apgar score of 9. The remaining two patients with positive CSTs at 34 and 36 weeks' gestation subsequently had reactive NSTs. At 36 and 38 weeks, respectively, both underwent cesarean section in labor for persistent late decelerations and cephalopelvic disproportion, respectively.



**Table III.** Pregnancy outcome in patients with a NRNST-positive CST by clinical management

| Item                   | Immature phospholipid profile | No labor | Labor          |                    | Total |
|------------------------|-------------------------------|----------|----------------|--------------------|-------|
|                        |                               |          | Fetal distress | No fetal distress* |       |
| Pregnancies (No.)      | 3                             | 8        | 12             | 18                 | 41    |
| Mode of delivery:      |                               |          |                |                    |       |
| Vaginal                | 1†                            | 0        | 1              | 15                 | 17    |
| Spontaneous            | 1                             | —        | —              | 13                 |       |
| Forceps                | —                             | —        | 1              | 2                  |       |
| Cesarean section       | 2                             | 8        | 11             | 3                  | 24    |
| Meconium               | 1                             | 3        | 6              | 3                  | 13    |
| Abnormal cord position | 2                             | 2        | 2              | 6                  | 12    |

\*Includes patient with positive CST at 28 and 36 weeks' gestation.

†IUFD.

Of the 38 pregnancies which were considered mature, either clinically or by phospholipid assay, all were delivered within 24 hours of the NR NST and positive CST. Of these, eight underwent direct cesarean section for the following indications: "uninducible" cervix (4), breech presentation (2), and repeat cesarean section (2) (Table III).

Of the 30 patients who were allowed to labor, which includes the pregnancy discussed earlier with positive CSTs at 28 and 36 weeks' gestation, direct FHR monitoring was applied in each case (Table III). Meconium-stained amniotic fluid was detected intrapartum in nine patients (30.0%). During labor, late decelerations of the FHR were observed in 18 (60.0%), and cesarean section for persistent late decelerations, despite position change and oxygen administration, was necessary in 10 instances (33.3%) (Table IV). In the patients with persistent FHR late decelerations, 80% (8 of 10) had absent or minimal FHR variability. One cesarean section for fetal distress was done because of sustained FHR bradycardia, and one patient developed late decelerations in the second stage of labor and was delivered by midforceps. At delivery, abnormal cord positions were recorded in eight instances (26.7%).

In summary, cesarean section was performed in 24 of 41 pregnancies (58.5%). Of those patients who labored, 11 of 30 required immediate cesarean section for fetal distress. Meconium-stained amniotic fluid and abnormal cord positions were present in 31.7% and 29.3%, respectively.

The fetal outcome for the entire NR NST-positive CST group are listed in Table V. While the overall incidence of growth-retarded babies was 29.2%, the fetal distress group had the highest rate of growth-retarded babies (75.0%). In addition, the fetal distress group had the highest incidence of neonatal complications and the lowest mean birth weight. The overall perinatal mortality rate was 73/1,000, with two neonatal deaths (Potter's syndrome, diaphragmatic hernia)

**Table IV.** Intrapartum FHR patterns in patients with induction of labor

| Item                                                           | Fetal distress | No fetal distress | Total |
|----------------------------------------------------------------|----------------|-------------------|-------|
| Pregnancies (No.)                                              | 12             | 18                | 30    |
| Decrease in baseline variability of <5 bpm                     | 8              | 3                 | 11    |
| Variable decelerations (severe)                                | 2              | 4                 | 6     |
| Late decelerations:                                            |                |                   |       |
| Mild                                                           | 2              | 5                 | 7     |
| Moderate                                                       | 5              | 1                 | 6     |
| Severe                                                         | 5              | 0                 | 5     |
| Combined late decelerations and decreased baseline variability | 8              | 0                 | 8     |

and one IUFD. Finally, congenital abnormalities were present in seven infants (17.1%) (Table VI).

#### Comment

Whenever a physician is faced with a patient who has a NR NST and a positive CST, the route of delivery is his primary concern. In most instances, these patients, as in this series, undergo antepartum FHR testing because of hypertensive disorders of pregnancy, postdates, or suspected IUGR.<sup>1-3</sup> Therefore, it is no surprise that the majority of the babies delivered are growth retarded. The incidence in various series ranges from 23%<sup>3</sup> to 41.4%.<sup>1</sup> However, the problem remains as to the best mode of delivery.

In clinical situations where the cervix is unfavorable for induction of labor, there is a breech presentation, or the patient has had a previous cesarean section, the decision to proceed directly to cesarean section is easy. Induction of labor, under these circumstances, would not be without the potential for additional maternal and fetal risk. As a result of this decision to proceed directly to cesarean section, a higher overall cesarean section rate which, depending on the series, ranges from 57.7% to 84%<sup>3</sup> is encountered in these cases.



**Table V.** Fetal outcome for entire NRNST population by clinical management

| Item                                     | Immature phospholipids | No labor | Labor          |                    | Total |
|------------------------------------------|------------------------|----------|----------------|--------------------|-------|
|                                          |                        |          | Fetal distress | No fetal distress* |       |
| Pregnancies (No.)                        | 3                      | 8        | 12             | 18                 | 41    |
| Apgar score (mean)                       |                        |          |                |                    |       |
| 1-minute                                 | 7.5                    | 6.0      | 6.5            | 7.9                | 7.0   |
| 5-minute                                 | 9.0                    | 7.9      | 8.1            | 8.8                | 8.4   |
| Birth weight (gm)                        | 3,238                  | 2,940    | 2,484          | 3,268              | 2,972 |
| Growth-retarded infants                  | 0                      | 2        | 9 <sup>A</sup> | 1                  | 12    |
| Neonatal complications (excluding death) | 1                      | 2        | 9              | 8                  | 20    |
| Perinatal deaths*                        | 1                      | 1        | 1              | 0                  | 3     |
| Congenital anomalies                     | 0                      | 3        | 2              | 2                  | 7     |

\*Includes patient with immature phospholipid profile at 28 weeks' gestation.

† $\chi^2 = 15$ ,  $p < 0.001$ .**Table VI.** Congenital abnormalities encountered in study group with NRNST-positive CST

| Abnormality            | No. |
|------------------------|-----|
| Hydrocephalus          | 1   |
| Microcephaly           | 1   |
| Cleft lip/palate       | 2   |
| Myelomeningocele       | 1   |
| Spina bifida           | 1   |
| Noncalcified calvarium | 1   |
| Diaphragmatic hernia   | 1   |
| Hydrocele              | 1   |
| Potter's syndrome      | 1   |

The major difficulties which arise in these cases occur when the fetus has been demonstrated to have pulmonary immaturity (L/S ratio  $< 2.0$  and absent phosphatidylglycerol at our institution) or the fetus is considered to have pulmonary maturity and the cervix is favorable for induction of labor. In those clinical situations where the fetus is determined to be immature, the decision to deliver that patient, in our opinion, should not rest solely on a single positive CST. As demonstrated by the 75% false positive CST rate that was observed in our immature group, a repeat CST within 24 hours appears to be warranted. This approach does not detract from the predictive value of the CST prior to 33 weeks' gestation<sup>9</sup> but does take into account this high false positive rate. If the repeat CST is positive, immediate delivery should be considered. While the adjunctive use of measurement of estriol levels in these patients would provide additional clinical information, the CST alone does provide adequate information about the status of the fetus in these patients.

In those patients with a NR NST-positive CST in whom labor was induced, intrapartum late decelerations were frequently observed in our investigation and in that of Gauthier and associates.<sup>6</sup> However, cesarean

section for repetitive late decelerations was necessary in only 33% of our laboring group (80% of these had decreased FHR variability) and in 20% of the patients of Gauthier and associates.<sup>6</sup> These results are in marked contrast to the results of Keegan and Paul<sup>10</sup> and of other published reports which deal with the positive CST alone.<sup>3-5</sup> Maternal positioning on the left side and intrapartum oxygen administration as well as marked uterine stimulation during the CST could account for this difference. More importantly, in those pregnancies which exhibit this antepartum FHR pattern, induction of labor should be considered where the capability for immediate cesarean section is available.

Growth-retarded infants have been frequently described in association with a positive CST<sup>3, 4</sup> and now with a NR NST-positive CST.<sup>6</sup> When these patients with this FHR pattern were allowed to labor, intrapartum fetal distress was found more often in the growth-retarded baby.<sup>3-6</sup> As a result, Sandenbergh and Odendaal<sup>11</sup> have recommended that primary cesarean section be performed in cases of severe IUGR. Support for this approach is also seen in our fetal distress group where 75.0% were growth retarded as compared with only 5.9% in the nondistress group ( $\chi^2 = 15$ ,  $p < 0.001$ ). Unfortunately, the diagnosis of IUGR is a difficult one to establish. Therefore, primary cesarean section should be considered in those instances where the diagnosis can be accurately made.

Neonatal complications and perinatal deaths were increased for this study group and were consistent with rates of other published reports.<sup>3-6</sup> However, the incidence of congenital abnormalities was high. In retrospect, a NR NST-positive CST would be in keeping with the intrapartum FHR patterns of anomalous fetuses described by Garite and associates.<sup>12</sup>

In conclusion, this investigation further substantiates the fetal and neonatal risks associated with a positive



CST. In those institutions with the capability for immediate cesarean section, an attempt at labor with direct FHR monitoring is justifiable. In those cases where IUGR can be accurately diagnosed, primary cesarean

section appears to be the procedure of choice. Finally, when this antepartum FHR pattern is observed, one should consider the possibility of an anomalous fetus.

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# FETUS, PLACENTA, AND NEWBORN

## Naloxone and its possible relationship to fetal endorphin levels and fetal distress

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This case report of fetal asphyxia and related studies suggest that fetal endorphins play a role in fetal pain tolerance and cardiovascular asphyctic responses. Elevated fetal endorphin levels may, like narcotics, produce abnormally "flat" fetal heart rate patterns intrapartum and fetal tolerance to pain. Although naloxone hydrochloride was used with some success to return flat fetal heart rate patterns to "normal," the unopposed antagonism of fetal endorphins by naloxone in times of fetal distress may be detrimental. (AM. J. OBSTET. GYNECOL. 139:16, 1981.)

NALOXONE HYDROCHLORIDE is considered to be a pure narcotic antagonist as it does not possess the agonistic actions of morphine-like drugs. Nor does it produce respiratory depression, psychotomimetic effects, or pupillary constriction. Thus, naloxone has been considered to have no pharmacologic activity in the absence of narcotics. Reported here are cases in which intrapartum naloxone had a probable effect upon human fetal heart rate responses in the absence of prior narcotic administration, presumably because naloxone inhibits fetal endorphins.

### Studies

In eight normal intrapartum fetuses at term who showed "flat" heart rate baselines (low beat-to-beat

variability) for at least 30 minutes, naloxone hydrochloride, 0.4 mg, was given intramuscularly to the mothers to arouse their fetuses. Four of the eight fetuses (50%) demonstrated a normal beat-to-beat variability within 20 minutes. Three mothers requested narcotics for pain relief within 20 minutes of the naloxone injection and, therefore, were excluded from the study. The remaining fetus (12%) demonstrated a flat baseline until late in the second stage of labor.

Following saline or placebo injection in five other fetuses with flat intrapartum fetal heart rate tracings, normal beat-to-beat variability returned in only one (20%) within 20 minutes. Either fetal scalp or umbilical artery pH was above 7.22 in all fetuses.

Based on statements that the drug had no detrimental fetal or neonatal effects,<sup>1, 2</sup> the use of naloxone hydrochloride under these circumstances had the approval of the hospital's Committee on Human Experimentation.

### Case history

S. N., a 19-year-old, para 0-0-0-0 black woman, had an uncomplicated antenatal course and was admitted in labor at term. Five hours after admission, a diagnosis of secondary arrest of labor was made when cervical dila-

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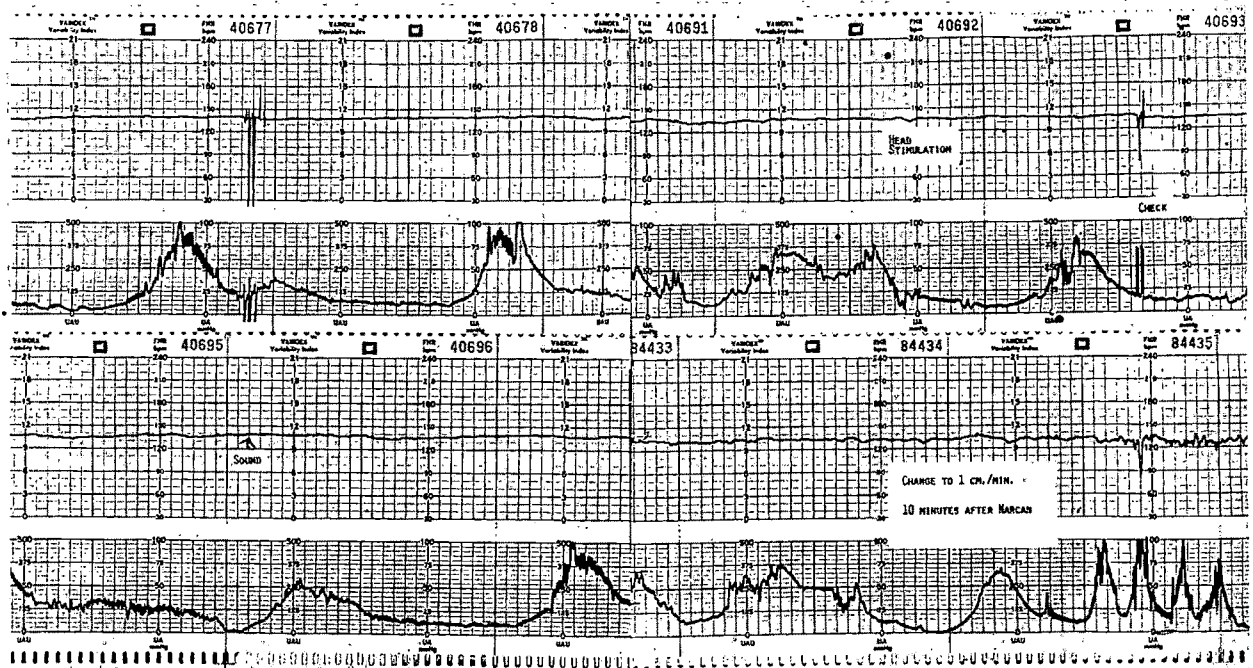


Fig. 1. Four sections of a fetal heart rate recording demonstrating loss of beat-to-beat variability. In the lower right recording, the paper speed was changed from 3 to 1 cm/min in order to better demonstrate beat-to-beat variability.

tion remained at 6 cm for 2 hours. An oxytocin infusion up to 16 mU/min was begun and the membranes were ruptured. Meconium staining of the amniotic fluid was noted, and the fetal heart rate patterns (No. 40677, Fig. 1) demonstrated a loss of beat-to-beat variability. An attempt to obtain a fetal scalp pH was unsuccessful. Because of the heart rate pattern and meconium staining of the fluid, preparations were made for a possible cesarean section and oxygen administration was begun.

As indicated in Fig. 1, attempts were then made to "arouse" the fetus (No. 40691 to No. 40696) by tactile stimulation of the fetal vertex and by an external sound stimulus over the vertex (100 dB, 2,000 Hz for 30 seconds). When these efforts to increase the fetal heart beat-to-beat variability failed, and 25 minutes after the oxytocin infusion was begun, the patient was given naloxone hydrochloride, 0.4 mg intramuscularly, to arouse her fetus. The nature and purpose of the drug had been explained to the patient. Ten minutes later, the fetal heart rate pattern was considered to show "normal" beat-to-beat variability (No. 84434).

Fifty minutes after the oxytocin infusion was begun, the cervix was fully dilated, and the vertex was at station +1; the patient began to push. For the first time "overshoots"<sup>3</sup> were noted in the fetal heart rate pattern (No. 8440 of Fig. 2). A fetal scalp pH obtained at this time was 6.97 and maternal "arterialized" venous blood pH was 7.39. Because the fetal heart rate pattern soon reverted to "normal," it was assumed that the scalp pH

was in error; nevertheless, the delivery was expedited. Frame No. 84449 of Fig. 2 was obtained in the delivery room immediately prior to application of the forceps. An easy midforceps delivery was accomplished, and the 3,350 gm female infant had a 1-minute Apgar score of 2. After resuscitation, the infant's 5-minute Apgar score was 5.

In the nursery, the infant appeared to be somewhat depressed and had signs and symptoms of meconium aspiration, although no meconium had been seen below the vocal cords at the time of delivery. The infant was maintained on a respirator with high settings, but at 22 hours of age, she began to have generalized seizures. These could not be controlled and she died at 37 hours of age of apparent respiratory failure. Post-mortem examination showed minimal meconium aspiration and no gross abnormalities of the brain. Death was presumed to be secondary to asphyxia in utero. The use of naloxone under these circumstances was discontinued after this case.

#### Comment

Hyperactive intrapartum fetal heart rate patterns may reflect increased arousal caused by fetal suffering or pain. Such fetal heart rate patterns, or even ominous patterns, may revert to normal with significant maternal systemic pain relief and/or anesthesia.<sup>4, 5</sup> Timor-Tritsch and associates<sup>6</sup> considered this apparent improvement in fetal heart rate patterns following



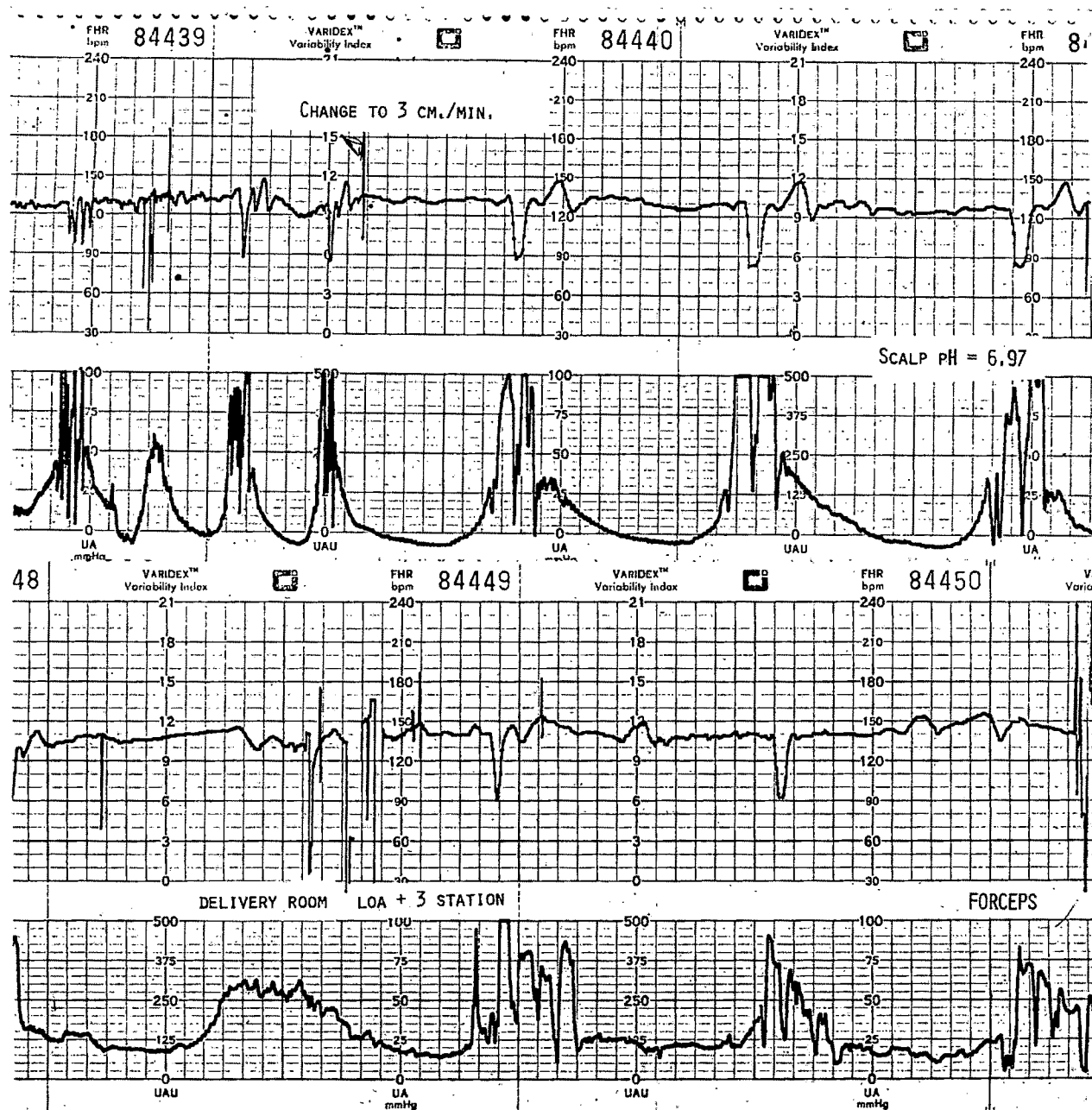


Fig. 2. Two sections of a fetal heart rate recording. In the upper section, "overshoots" are seen (paper speed changed back to 3 cm/min). Lower recording was obtained immediately prior to forceps application and was considered to demonstrate no abnormalities. Umbilical vein pH was 7.20. Artery pH was 7.07.

general anesthesia to represent decreased intensity of uterine contractions. However, another interpretation is that the well-being of the intrapartum fetus subjected to severe pain, just as in the suffering adult, is improved by analgesia.<sup>4, 5</sup>

Narcotics offer a small degree of fetal protection from asphyxia,<sup>4, 5</sup> and endogenous endorphins or other self-produced opiates may serve a like function

for the stressed fetus. Gautray and associates<sup>7</sup> found increased endorphins in amniotic fluid during labor, especially in the case of fetal distress. Wardlaw and associates<sup>8</sup> observed increased plasma beta endorphin levels in fetal sheep after hypoxia and, in human fetuses, described a negative correlation between umbilical artery pH and fetal beta endorphin-like immunoactivity. They concluded that hypoxia and secondary



acidosis may be a stimulus for the release of fetal endorphins. Despite numerous studies suggesting that naloxone is without effect in the absence of prior narcotic administration,<sup>1, 2</sup> the possibility exists that antagonism of endorphins by naloxone may be detrimental to some distressed fetuses. In single case reports, naloxone has been associated with hypertension in adults.<sup>9, 10</sup>

Naloxone hydrochloride is considered to antagonize endorphins as well as narcotics. In adult humans, it has been shown that placebo-induced tolerance to chronic pain is reduced by naloxone injection.<sup>11</sup> This increased pain sensitivity presumably reflects naloxone antagonism of the endorphins produced in response to chronic pain. Human fetuses and their placentas have varying amounts of endorphin activity,<sup>8, 12</sup> which could explain their different intrapartum fetal heart rate reactivities, which may likewise be increased by naloxone. The intrapartum fetal heart rate pattern with low beat-to-beat variability (reactivity) could reflect a high fetal endorphin level, which allows the fetus to "sleep" during labor. Such fetal heart rate patterns when associated with decelerations are sometimes of medical concern and it seems reasonable to attempt to arouse the fetus to confirm its well-being. This modest success of obtaining "normal" fetal heart rate patterns after naloxone administration supports the hypothesis that elevated fetal endorphin levels contribute to fetal heart rate patterns of loss of beat-to-beat variability.

In the case of S. N., no scalp pH was obtained at the time of the initial discovery of decreased fetal heart beat-to-beat variability. None of the other fetuses with

loss of beat-to-beat variability had abnormal pHs, and because our prior experience suggested that a flat fetal heart rate baseline without decelerations was not associated with acidemia, plans for vaginal delivery seemed reasonable. Zabor and Quilligan<sup>13</sup> similarly have found that loss of beat-to-beat variability without other fetal heart rate abnormalities is seldom associated with fetal acidemia. If one assumes that there was no acidemia in this fetus at the time of the naloxone administration and blockage of fetal endorphins, naloxone may have both enhanced fetal asphyxia and caused the appearance of "normal" fetal heart rate patterns. Obviously, given the uncertainties of this particular case report, it cannot be proved that naloxone was detrimental to this fetus for, indeed, it is not even known for certain that naloxone effectively crosses the placenta, although clinical experience suggests that it does.<sup>14</sup> Unrelated to these concepts, the Committee on Drugs (with the approval of the American Academy of Pediatrics and the American College of Obstetricians and Gynecologists) has stated (1980) that naloxone is not recommended for administration to the mother just prior to delivery to reverse the fetal and neonatal effects of maternally administered narcotic analgesics.<sup>15</sup>

The psychiatric literature suggests that birth is a painful process for the fetus<sup>16</sup> and may be a source of stress. The purpose of this report is to support the concept that fetuses may endure pain during labor, that endorphins or similar self-produced opiates aid the fetus in withstanding such stress, and that agents like naloxone may be detrimental to fetal welfare through counteracting their endorphins.

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# Maternal and fetal concentrations of morphine after epidural administration during labor

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In this study of 20 healthy women in labor we attempted to determine the efficacy of epidural morphine as an obstetric analgesic. Four patients experienced some relief of pain while 16 patients reported no effect on pain. One newborn infant demonstrated possible morphine-induced respiratory depression. It was concluded that morphine is ineffective as an obstetric analgesic. (AM. J. OBSTET. GYNECOL. 139:20, 1981.)

RECENTLY, INTEREST has been focused on administration of extradural injections of morphine for relief of acute and chronic pain. For obstetric use this method could be most interesting, but in a recent report by Husemeyer and associates,<sup>1</sup> poor analgesic effect of epidural morphine (2 mg) was noted.

## Material and methods

Our subjects consisted of 20 healthy women in labor (gestational weeks 36 to 43). For analgesia during the first stage of labor, 4 to 6 mg of morphine diluted in 0.9% saline to a total volume of 10 ml was given via an epidural catheter inserted at T11-12 or T12-L1. Four of the 20 patients experienced some alleviation of pain. The other 16 patients reported no amelioration of pain, and bupivacaine (5 to 6 ml, 0.25%) was given to 13 patients via the epidural catheter for adequate analgesia.

Among the 20 newborn infants, one (5 mg of morphine given to the mother 1 hour and 50 minutes before delivery) demonstrated respiratory depression

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**Table I.** Injection-delivery times related to concentrations of morphine

| Injection-delivery time (hours) | Morphine concentration (ng/ml) |        |
|---------------------------------|--------------------------------|--------|
|                                 | Mother                         | Infant |
| 0.5                             | 10.6                           | 11.8   |
| 1.0                             | 9.6                            | 9.3    |
| 1.0                             | 9.7                            | —      |
| 1.5                             | 7.1                            | 4.9    |
| 3.0                             | 5.9                            | 5.9    |
| 3.0                             | 4.4                            | 4.0    |
| 3.0                             | 3.3                            | 3.7    |
| 3.5                             | 1.8                            | 2.5    |
| 4.0                             | 2.8                            | 3.9    |
| 5.5                             | —                              | 1.3    |
| 6.0                             | 1.3                            | 1.3    |
| 6.0                             | 1.3                            | 1.1    |
| 7.0                             | —                              | 2.0    |
| 7.5                             | 0.5                            | 1.1    |
| 10.0                            | 1.4                            | 1.8    |

immediately after a normal delivery (gestational week 36, birth weight 2,670 gm). Recordings of heart rate and breathing (3 to 30 minutes after delivery) showed repeated periods of apnea during the first 16 minutes. Morphine-induced respiratory depression could not be excluded. This case, which occurred at the beginning of the study, initiated analysis of morphine in maternal and cord blood at delivery. A total of 27 samples were obtained. Morphine was analyzed by a gas-liquid chromatographic method.<sup>2</sup>

Delivery occurred within 0.5 to 10.0 hours after administration of morphine. The highest morphine con-



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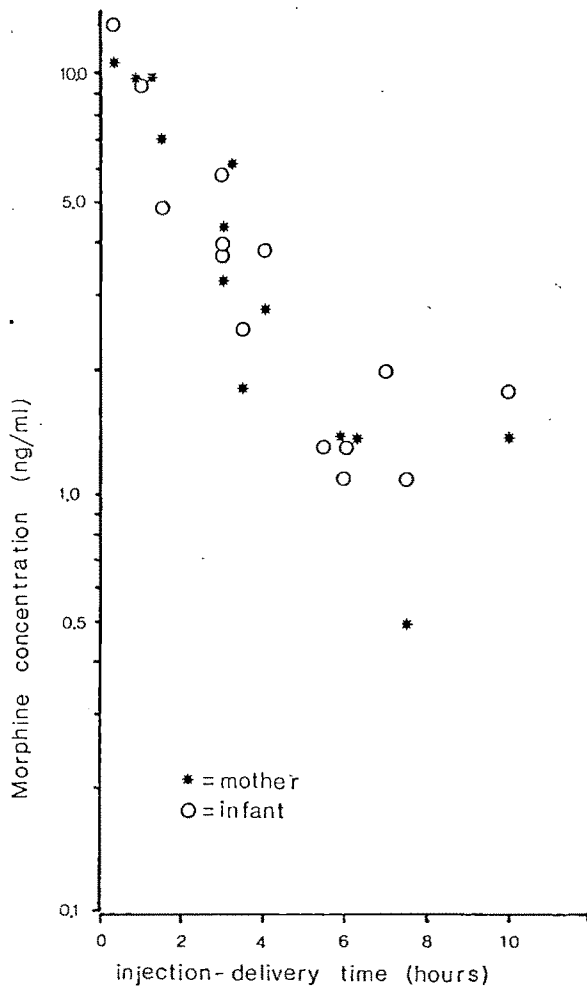


Fig. 1. Relationship between morphine concentrations and injection-delivery time.

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centrations in the mother and fetus were found in those with the shortest time interval between injection and delivery. These findings indicate a rapid absorption of morphine from the epidural space and rapid transport across the placenta (Table I). Furthermore, there was a close correlation between maternal and fetal concentrations. The apparent relation between morphine concentrations and injection-delivery times (Fig. 1) suggests redistribution and degradation of the drug in both mother and fetus.

#### Results and comment

The analgesic effect of extradurally administered morphine (4 to 6 mg) during labor was poor. In fact, it was not better than the effect reported earlier,<sup>1</sup> despite a dose that was two to three times higher.

Epidurally administered morphine does not seem to have potential as an alternative drug in obstetric analgesia. Furthermore, the rapid transport of morphine from the epidural space across the placenta implies a potential risk to the fetus. With a short injection-delivery time, high doses, and prematurity, this risk might be of clinical importance.



# Effect of endogenous insulin release on fetal alanine concentration and uptake

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Insulin secretion in the fetus may be important in the modulation of selected fetal amino acid concentration and uptakes. To test this hypothesis we observed the changes in fetal alanine concentrations and uptake after tolbutamide-induced insulin release in the fetal lamb. The basal umbilical venous-arterial alanine difference was  $29.1 \pm 4.2$   $\mu\text{moles/L}$ . Fetal alanine uptake was  $5.1 \pm 0.6$   $\mu\text{moles/kg/min}$ . After tolbutamide infusion fetal insulin concentration rose fourfold by 30 minutes. Fetal glucose concentration fell to approximately 75% of control values. Both arterial and umbilical venous alanine contents fell significantly ( $p < 0.01$ ), yet the fetal alanine venous-arterial difference also fell significantly ( $p < 0.05$ ) by 30 minutes. Fetal alanine uptake from the pooled experiments did not change significantly after tolbutamide. When only insulin responses of less than 150  $\mu\text{U/ml}$  were considered, however, a significant ( $p < 0.02$ ) fall in alanine uptake was noted. Two injections in one animal caused peak insulin levels above 150  $\mu\text{U/ml}$ . Although limited, data from these latter experiments suggested an increase in fetal alanine uptake at these higher insulin concentrations. Thus, endogenous fetal insulin release caused a significant hyperinsulinism and consequent hypoglycemia. The fetal hypoalaninemia produced may have been due to a number of factors, including an acute decrease in placental alanine transfer and decreased fetal hepatic gluconeogenesis. A differential effect of high versus low insulin responses upon fetal alanine uptake is also suggested. (AM. J. OBSTET. GYNECOL. 139:22, 1981.)

BOTH ENDOGENOUS INSULIN release<sup>1</sup> and exogenous insulin infusion<sup>2</sup> have induced marked alterations in concentration and uptake of selective amino acids in vivo and in vitro adult preparations. It has been suggested<sup>3</sup> that insulin may play a pivotal role in the acceleration of anabolic processes in the adult via its ability to enhance both amino acid uptake and protein synthesis in muscle and liver. Consonant with this belief is the observation that amino acids are potent insulin secretagogues for the adult.<sup>4</sup>

The factors involved in the control of fetal growth

are unclear, particularly those related to amino acid uptake and accretion. Certain amino acids such as alanine, glycine, and leucine are taken up in significant quantities by the fetus.<sup>5</sup> Alanine, when infused into the fetal lamb, has produced a significant biphasic insulin response,<sup>6</sup> similar to that seen in the adult. What effect insulin may have upon fetal amino acid metabolism is unclear, however. With the use of the sulfonylurea tolbutamide, a potent insulin secretagogue in the fetus<sup>7</sup> as well as the adult,<sup>1</sup> we have sought to explore the relationship between endogenous fetal insulin release and changes in alanine uptake in the chronically catheterized fetal lamb.

## Material and methods

**Surgical preparation.** Five pregnant ewes between 125 and 140 days' gestational age (term gestation is 147 days) were studied. Preoperatively all animals received intravenous sodium pentobarbital and atropine as well as a spinal anesthetic. Indwelling polyvinyl chloride catheters were placed in the umbilical vein, fetal pedal artery, two fetal pedal veins, and the maternal femoral artery for the purposes of sampling and infusion.

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Catheters were then tunneled subcutaneously to a pouch on the mother's flank. Postoperative care and feeding were as previously described.<sup>7</sup> All animals were allowed at least a 4-day recovery period before experiments were begun. All animals were feeding ad libitum by this time.

**Experimental design.** Thirteen infusions of tolbutamide were performed in the five experimental animals. In those animals in which serial infusions were performed, at least 48 to 72 hours separated the exposure to tolbutamide. During a 30-minute control period fetal blood samples were withdrawn twice for determination of umbilical venous and arterial alanine content, as well as arterial glucose and insulin concentration. Umbilical blood flow was assessed by means of the antipyrine steady-state diffusion method.<sup>8</sup> One fetal venous catheter served for antipyrine infusion. Tolbutamide, in a dose of 100 to 200 mg/kg estimated fetal weight, was infused over 1 minute through the other fetal venous catheter at the beginning of the 45-minute experimental period. Fetal blood was withdrawn at 30 and 45 minutes from the arterial and umbilical venous catheters to assess whole-blood alanine and antipyrine content as well as arterial glucose and insulin concentrations. These times were chosen empirically from previous work assessing changes in peak fetal glucose uptake after tolbutamide.<sup>9</sup>

Fetal uptake of alanine ( $Q_{ala}$ ) was calculated by the Fick equation:

$$Q_{ala} (\mu\text{moles/kg} \cdot \text{min}) = \frac{\text{alanine venous-arterial difference } (\mu\text{moles/ml}) \times \text{umbilical blood flow (ml/kg} \cdot \text{min)}}{\text{alanine venous-arterial difference } (\mu\text{moles/ml}) \times \text{umbilical blood flow (ml/kg} \cdot \text{min)}}$$

Fetal weight at the time of the experiment was estimated by extrapolation from the known birth weight by means of fetal sheep growth charts. Fetal umbilical venous pH,  $PCO_2$ , and  $PO_2$  as well as maternal glucose and insulin were also assessed in the control and experimental periods to note possible deleterious effects of tolbutamide on maternal and fetal homeostasis.

**Biochemical analyses.** Blood samples for glucose and alanine were withdrawn and placed in separate test tubes coated with ethylenediaminetetra-acetic acid. Whole-blood glucose was measured by means of the glucose oxidase technique. Whole-blood alanine specimens were frozen at  $-20^\circ\text{C}$  and then thawed for batch assay. The whole-blood alanine assay was performed according to the alanine dehydrogenase method<sup>6</sup> after deproteinization with 5% perchloric acid. A recovery of  $108\% \pm 2\%$  has been noted in our laboratory with this assay. Antipyrine concentration was determined by means of a Technicon AutoAnalyzer. Insulin concentration was measured by insulin radioimmunoassay as previously described.<sup>7</sup>

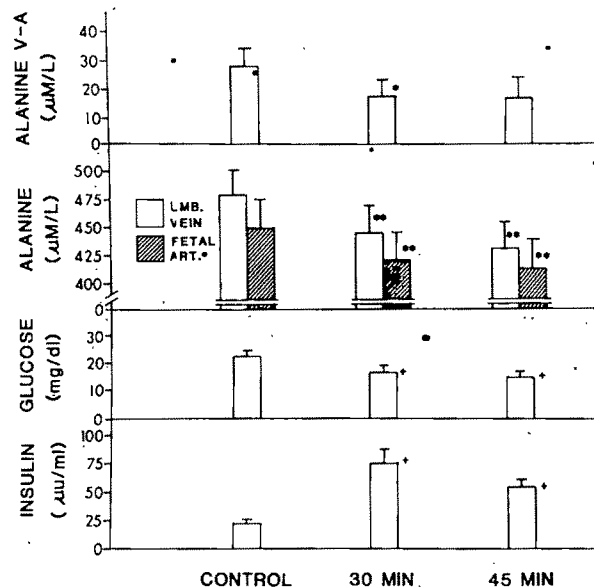


Fig. 1. Response of five fetal lambs to tolbutamide infusion (\* =  $p < 0.05$ ; \*\* =  $p < 0.01$ ; + =  $p < 0.001$ ).

**Statistical methods.** All results are expressed as mean  $\pm$  SEM. The paired Student's  $t$  test was used to determine changes in insulin, glucose, and alanine concentrations as well as alterations in umbilical flow and fetal venous-arterial difference and uptake of alanine.

## Results

Of the five animal preparations, 4 ewes delivered healthy term lambs. One in utero death occurred 5 days after tolbutamide infusion (the only twin preparation) and appeared unrelated to the experimental procedure. No significant changes were noted in fetal umbilical venous pH,  $PCO_2$ ,  $PO_2$ , or oxygen content after tolbutamide, nor were significant changes apparent in maternal glucose or insulin during the experiments. Basal whole-blood alanine concentrations are depicted in Fig. 1 ( $480 \pm 28$  and  $449 \pm 26$   $\mu\text{moles/L}$  for the umbilical vein and artery, respectively). The whole-blood alanine venous-arterial difference was assessed in 23 separate control specimens as  $29.1 \pm 4.2$   $\mu\text{moles/L}$ . As shown in the table, this corresponds to a basal umbilical alanine uptake of  $5.1 \pm 0.6$   $\mu\text{moles/kg/min}$ .

Thirty minutes after tolbutamide infusion, the fetal arterial insulin concentration had risen almost fourfold (Fig. 1,  $p < 0.001$ ) and remained significantly elevated at 45 minutes. Fetal arterial glucose content fell from  $21.4 \pm 2.2$  mg/dl to  $15.6 \pm 2.3$  and then  $14.1 \pm 1.8$  mg/dl by 30 and 45 minutes, respectively (both significantly different from basal,  $p < 0.001$ ). This represents a fall of approximately 25% from baseline glucose



Table I. Effect of tolbutamide on fetal alanine uptake

|                                               | Control         | 30 min                      | 45 min                      |
|-----------------------------------------------|-----------------|-----------------------------|-----------------------------|
| Alanine venous-arterial difference (μmoles/L) | 29.1 ± 4.2 (22) | 17.3 ± 5.7 (12)<br>p < 0.05 | 17.2 ± 5.7 (13)<br>p = 0.07 |
| UBF (ml/kg/min)                               | 174 ± 8 (12)    | 163 ± 7 (12)<br>NS          | 152 ± 8 (12)<br>p < 0.01    |
| Qala (μmoles/kg/min)                          | 5.1 ± 0.6 (12)  | 3.6 ± 1.1 (11)<br>NS        | —                           |
| Qala, (high insulin deleted) (μmoles/kg/min)  | 5.1 ± 0.7 (10)  | 2.4 ± 0.9 (9)<br>p < 0.02   | —                           |

NS: Not significant.

concentration. Changes in fetal alanine concentration are depicted in Fig. 1 as well. Associated with the elevation of plasma insulin, both arterial and umbilical venous alanine concentrations fell, reaching nadirs by 45 minutes after infusion ( $37.8 \pm 11.9$  and  $50.9 \pm 10.2$  μmoles/L below baseline, respectively.). A significant ( $p < 0.05$ ) fall in venous-arterial alanine difference was noted at 30 minutes after infusion. Although the venous-arterial difference remained low at 45 minutes, statistical significance was not achieved ( $p = 0.07$ ).

Although fetal glucose and alanine concentrations both fell after endogenous insulin release, no linear relationship existed between the absolute decrements of one versus the other. Also no relationship was apparent between the degree of change of fetal arterial, umbilical venous, or venous-arterial difference of alanine and peak insulin level achieved.

The mean changes in fetal alanine uptake are shown in Table I. Mean umbilical blood flow (UBF) in the control period was  $174 \pm 8$  ml/kg/min and did not change significantly by 30 minutes. Mean fetal alanine uptake fell to 70% of control values, however, by 30 minutes after infusion. Because of the large degree of interanimal variability, this change was not statistically significant. UBF thereafter fell approximately 12% below baseline, thus invalidating the use of antipyrine in estimation of UBF and alanine uptake at 45 minutes. That this was not due to instability of the preparation is confirmed by the normality of control and 45-minute umbilical venous blood gas values and oxygen contents in all infusion experiments.

No linear relation was apparent between the changes in alanine uptake at 30 minutes and insulin concentration. However, in the only animal studied four times with differing doses of tolbutamide, it was noted that when peak insulin levels exceeded 150 μU/ml, an increase rather than a decrease in umbilical alanine uptake was apparent. These were the only two in a total of 13 infusions in which insulin levels rose above a concentration of 150 μU/ml. If these two infusions are deleted from the umbilical uptake data, a highly sig-

nificant fall in Qala is apparent at 30 minutes after infusion (Table I). This may suggest a differential effect of large and small increments of insulin upon alanine uptake.

Comment

The sulfonylurea tolbutamide has been used in the adult to explore the effects of endogenous insulin release upon glucose and amino acid metabolism. In previous work we have documented that tolbutamide infusion into the fetal lamb is associated with transient but significant fetal hyperinsulinism and hypoglycemia,<sup>7</sup> as occurs in the adult. The hypoglycemia in the fetus appears to be due to an increase in fetal glucose uptake noted particularly 30 minutes after infusion.<sup>9</sup>

Both tolbutamide and exogenous insulin infusions in adult humans are associated with a fall in peripheral amino acid concentration,<sup>1, 2</sup> probably related to insulin's ability to increase cellular protein synthesis in liver and muscle.<sup>3</sup> The data regarding changes in individual amino acid concentrations, however, are confusing, particularly with respect to alanine. Pozefsky and associates<sup>2</sup> found that insulin did not enhance adult peripheral muscle alanine uptake. In contrast, Rannels and associates,<sup>10</sup> using the adult rat heart found increases in alanine uptake after insulin stimulation. Adibi and co-workers<sup>1</sup> found a significant fall in the plasma concentration of a number of amino acids, particularly alanine, following tolbutamide-stimulated insulin release in normal human adult subjects.

Little evidence is available concerning the role of insulin in the control of amino acid utilization by fetal tissues. Fricke and Clarke<sup>11</sup> have demonstrated insulin-stimulated acceleration of alpha amino isobutyric acid (AIB) transport in the fetal diaphragm and heart and at least some fetal tissues such as the heart<sup>12</sup> appear to utilize the same transport pathway for alanine as for AIB. Picon<sup>13</sup> has also shown that long-term fetal insulin administration is associated with an increase in nitrogen content in the rat fetus, suggesting an increase in protein synthesis. Schwartz and Rall<sup>14</sup> have shown that

in the presence of exogenous insulin, a marked diminution in fetal hepatic glucose production from alanine is evident. Thus, insulin might theoretically accelerate fetal growth by promoting peripheral amino acid uptake and protein synthesis as well as inhibiting hepatic conversion of alanine to glucose.

Since alanine is a pivotal amino acid in the hepatic production of glucose, we sought to evaluate the effects of endogenous insulin release on fetal alanine metabolism. We have confirmed the data of Lemons and co-workers<sup>5</sup> regarding the resting umbilical venous-arterial alanine difference and have also directly measured its uptake by the fetus. This uptake corresponds with approximately 7% of the net umbilical nitrogen uptake as estimated by Lemons and associates. These authors suggest that greater than 50% of the fetal alanine uptake is utilized for purposes other than accretion. In contrast to our data, the total uterine uptake of alanine has recently been measured in the ewe.<sup>15</sup> Near term this uptake is less than 40% of the fetal alanine uptake noted in our experiments. Methodologic differences may account for this discrepancy but it is equally likely that placental interconversion and production of alanine may be contributory.

Tolbutamide-induced endogenous insulin release in the fetal lamb is associated with a fall in both fetal whole-blood arterial and umbilical venous alanine levels. At insulin concentrations below 150  $\mu\text{U}/\text{ml}$  a fall in the umbilical venous-arterial difference corresponded to a greater than 50% reduction in fetal alanine uptake at 30 minutes after infusion. Although hypoalaninemia was still present at 45 minutes, alanine uptake could not be calculated because of a small but significant fall in UBF. The reasons for the fall in UBF are unclear but did not appear to be related to instability or deterioration of the fetal preparations.

The data regarding alanine uptake in the presence of high insulin levels are limited since tolbutamide-stimulated insulin release in the fetal lamb rarely

exceeds a maximum concentration of 100  $\mu\text{U}/\text{ml}$ .<sup>7</sup> Unfortunately, in our series of experiments, only two injections in one animal resulted in fetal insulin concentrations exceeding this value. This limited information, however, would suggest that in the ovine fetus large elevations in plasma insulin may cause an increase in fetal alanine uptake while lower increments of hyperinsulinism may result in a fall in fetal alanine uptake. This would support the findings of Riggs and associates<sup>16</sup> that fetal muscle amino acid transport is stimulated by, but relatively resistant to, insulin administration. A fall in fetal alanine uptake at low insulin concentration could be due primarily to a decrease in fetal alanine catabolism in either liver or muscle. However, the larger fall in umbilical venous versus arterial alanine concentration suggests an acute decrease in placental alanine production or transfer.

It is unlikely that tolbutamide itself could have exerted such acute effects. Sulfonyleurea administration has been associated with a variety of peripheral effects, including a decrease in the rate of protein synthesis.<sup>17</sup> These effects are only evident, however, after chronic administration. There is presently no evidence to suggest that the acute hypoglycemic or hypoaminoacidemic effects of tolbutamide act by a pathway other than acute hyperinsulinism.

In summary, we have documented a significant basal uptake of alanine in the fetal sheep. Tolbutamide-induced insulin release below a concentration of 150  $\mu\text{U}/\text{ml}$  in the fetus caused an abrupt fall in fetal alanine concentration as well as fetal uptake but the reasons for this are unclear. This fall, coupled with the increase in fetal glucose uptake seen after tolbutamide, is consistent with the view that insulin may encourage fetal growth via its stimulation of the utilization of glucose as the major fuel substrate. The role of endogenous insulin release in alteration of placental alanine transfer and fetal carcass uptake is unclear.

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# Fetal macrosomia in experimental maternal diabetes

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Fetal macrosomia in diabetic pregnancy has been shown to accompany increases in the fetal levels of deoxyribonucleic acid (DNA) and proteins in animal model systems. In order to elucidate the underlying mechanisms of this macrosomia, the synthesis of DNA and proteins, the transport of the precursors, and the tissue level of DNA polymerase activities in macrosomic fetuses from mildly diabetic rats were compared with those in normal fetuses on 20.5 and 19.5 days of gestation. Increases in the influx of precursors and stimulation of synthesis of the macromolecules were observed in macrosomic fetuses as early as 19.5 days of gestation. However, stimulation of DNA polymerase activities in macrosomic fetuses did not occur until 20.5 days of gestation. Therefore, stimulation of DNA polymerase may participate in maintenance of macrosomia but does not initiate macrosomia. Whether or not increases in both the influx of substrates and the synthesis activities precede the macrosomia has yet to be determined. (AM. J. OBSTET. GYNECOL. 139:27, 1981.)

ALTHOUGH FETAL MACROSOMIA induced by maternal diabetes has long been recognized in human pregnancy,<sup>1, 2</sup> biochemical mechanisms underlying the pathogenesis and consequences of macrosomia have not been elucidated. One reason for the slow progress in the study of the mechanisms is the lack of an adequate animal model system. In animal model systems the experimental results concerning the fetal weight in diabetic pregnancy have been controversial until recent years.<sup>3, 4</sup> Evidence obtained very recently in our laboratories and other laboratories has finally established that neonatal or fetal macrosomia in animal model systems is produced only under mildly diabetic conditions of the mother and the macrosomia disappears as the maternal diabetes worsens.<sup>5-7</sup> Moreover, we have observed that the maternal diabetes-mediated changes in neonatal weight accompany corresponding changes in the deoxyribonucleic acid (DNA) content and DNA polymerase activities in the neonatal tissue.<sup>5</sup> This observation implicates DNA polymerase activities as a

possible initiating cause for the stimulation of DNA synthesis and thereby manifestation of fetal macrosomia in diabetic pregnancy. In the present study, the time courses of changes in the fetal contents of DNA and proteins, precursor incorporation into these macromolecules, and tissue levels of DNA polymerase activities were determined and their relationships to fetal macrosomia were examined.

## Methods

**Animals.** Female Sprague-Dawley rats weighing 180 to 200 gm were divided into normal and streptozotocin-treated groups; animals in the streptozotocin-treated groups were given 32 mg/kg of streptozotocin intraperitoneally as described previously.<sup>5</sup> On the tenth day thereafter blood samples were obtained from tail veins and the glucose concentration was estimated by the method of Jarrett and associates.<sup>8</sup> Those streptozotocin-treated animals in which the blood glucose level was in the range of 130 to 300 mg/100 ml were termed mildly diabetic. Both normal and mildly diabetic animals were given Purina Rat Chow and water ad libitum and subsequently were used for mating.

A group of 10 female rats weighing 200 to 250 gm were housed in one cage, and one male rat was placed in the cage from 1700 to 0800 hours. The animals were weighed and inspected every morning between 0800 and 0900 hours. The presence of sperm on vaginal smears indicated mating, and the gestational period at this time was considered 0.5 day. In order to eliminate

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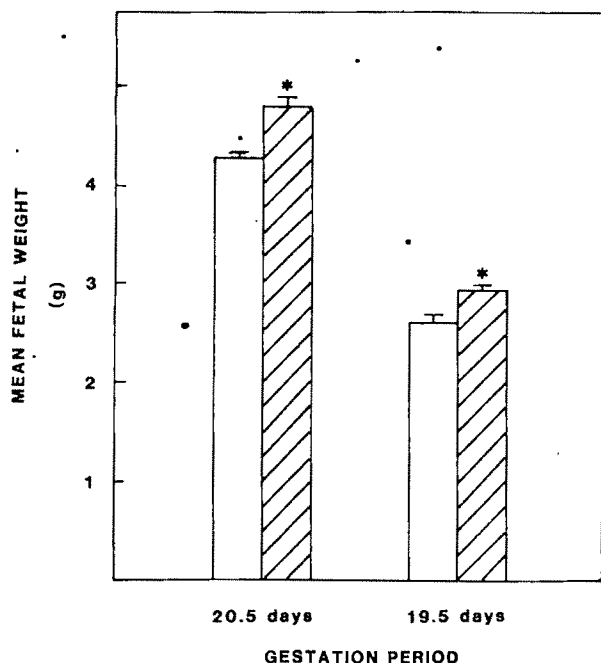


Fig. 1. Mean body weights of fetuses from normal and mildly diabetic mothers. Fetuses were collected from normal (*open bars*) and mildly diabetic (*shaded bars*) mothers on days 20.5 and 19.5 of gestation. Each of the four groups consisted of 15 litters, and the height of the bar indicates the mean value of average fetal weights from 15 litters with the standard error of the mean. Asterisks show  $P < 0.05$  when compared with the corresponding mean normal fetal weight.

possible variation in fetal sizes due to causes other than maternal diabetes, the following precautionary measures were implemented: One male rat was used for mating both normal and diabetic groups of rats in order to obtain matching litters for a set of experiments; female rats were eliminated from the study if they failed to mate before their body weight reached 250 gm; only those litters containing 10 to 12 fetuses were included in the study.

Fetuses from normal and mildly diabetic mothers were removed at 20.5 and 19.5 days of gestation. A total of 15 litters of fetuses from normal mothers were collected on day 20.5 of gestation, and the mean value of the average fetal weights of littermates and the standard error of the mean value were calculated.

Matching litters from mildly diabetic mothers were then obtained, and only those litters which had an average fetal weight greater than the normal mean fetal weight by two times the standard error were selected. After all the experiments for the 20.5-day fetuses were completed, the 19.5-day fetuses were evaluated in the same manner.

In those animals used in the radioisotope tracer ex-

periments, either 20  $\mu\text{Ci}/100\text{ gm}$  of  $^3\text{H}$ -thymidine, (50 Ci/mmol, New England Nuclear) or 10  $\mu\text{Ci}/100\text{ gm}$  of  $^3\text{H}$ -L-leucine (70 Ci/mmol, New England Nuclear) was injected intraperitoneally into the side of the mother exactly 120 minutes before the animal was killed.

The pregnant rat was killed by decapitation and each fetus was isolated. The yolk sack and umbilical cord were removed, and the fetus was then rinsed in a cold buffer solution containing the following: Tris-hydrochloric acid, 37.0 mM (pH 7.3); potassium chloride 50.0 mM; magnesium chloride, 3.3 mM; ethylenediaminetetraacetic acid, 0.5 mM; 2-mercaptoethanol, 6.0 mM. After blotting to remove the excess buffer solution, the fetuses from each litter were counted and weighed in order to determine the average fetal weight of the litter. The fetuses were killed and homogenized in a Polytron homogenizer for 1 minute. The final concentration was adjusted with the buffer solution to 1 gm of fetus per 10 ml of the total homogenate. This total homogenate was used as a starting material for the following experiments.

**Determination of fetal DNA and proteins.** The concentrations of DNA and proteins in the total homogenate were estimated by the methods of Schneider<sup>9</sup> and Lowry and associates,<sup>10</sup> respectively. From these results the contents of these macromolecules (milligrams per fetus) were calculated.

**Measurement of  $^3\text{H}$ -thymidine and  $^3\text{H}$ -leucine.** Aliquots of the total homogenate were solubilized in equal volumes of 0.05N sodium hydroxide solution, and 0.2 ml of solubilized sample was placed in a scintillation counting vial. The radioactivity was measured in a Beckman CPM 100 scintillation counter.<sup>11</sup> From these results the amount of radioactive precursors in the total pool (counts per minute of  $^3\text{H}$ -thymidine or  $^3\text{H}$ -L-leucine per fetus) was estimated.

From 3 ml aliquots of the total homogenate, DNA and proteins were extracted in triplicate by the methods<sup>9, 12</sup> described previously, and the amounts of radioactivity in DNA (counts per minute of  $^3\text{H}$ -thymidine/DNA/fetus) or in proteins (counts per minute of  $^3\text{H}$ -L-leucine/proteins/fetus) were measured in the same manner as that described above.

**DNA polymerase- $\alpha$  activity assay.** The polymerase activity was assayed by the method of Bollum<sup>13</sup> with the following modifications: The total homogenate was centrifuged at  $10,000 \times g$  for 20 minutes, and the supernatant fraction was centrifuged again at  $105,000 \times g$  for 60 minutes. The  $105,000 \times g$  supernatant fluid was used without dilution for estimating the enzyme activity. The enzyme activity was determined by mea-

asuring the polymerization of  $^3\text{H}$ -thymidine triphosphate (TTP) (50 Ci/mmol; New England Nuclear) into the acid-insoluble fraction in the presence of activated calf thymus DNA as the template. The assay medium contained 50  $\mu\text{M}$  each of d-adenosine triphosphate, d-guanosine triphosphate, and d-cytidine triphosphate, 75  $\mu\text{M}$   $^3\text{H}$ -TTP (20  $\mu\text{Ci}/\text{mmole}$ ), and 0.06 mg/ml of activated DNA in the buffer solution described above. A 50  $\mu\text{l}$  sample of the  $105,000 \times \text{g}$  supernatant fluid was placed in an aliquot of 200  $\mu\text{l}$  of the assay medium, and this reaction mixture was incubated at  $37^\circ\text{C}$  for 60 minutes. At zero time and at the end of the 60-minute incubation period, 25  $\mu\text{l}$  aliquots of the reaction mixture were withdrawn in duplicate and applied to filter paper disks, which were washed and dried according to the procedure described by Bollum.<sup>13</sup> The treated disks were placed in individual vials and the radioactivity was measured by the method described previously.<sup>11, 12</sup> The amount of radioactive TTP incorporated into the acid-insoluble material (counts per minute of  $^3\text{H}$ -TTP polymerized per soluble protein in 1 gm of fetal tissue) is a measure of the polymerase activity.

**Comparison of macrosomic fetus with its normal littermates.** Fetuses in four litters removed from diabetic mothers on day 19.5 of gestation were used for comparison of macrosomic fetuses and normal littermates with respect to the parameters determined in the preceding experiments. In this series of experiments, individual fetal weights were measured, and those fetuses whose weights were larger than the mean normal fetal weight by two times the standard error (both values established in the preceding experiments) were selected as macrosomic fetuses and were processed separately from the remaining littermates. Other experimental procedures were the same as those described above. Statistical analysis was not performed on the results of these experiments for obvious reasons.

**Statistics.** Throughout this report, data were expressed in mean  $\pm$  standard error whenever applicable, and significances of differences between mean values being compared were determined by the paired  $t$  test. The difference was considered significant when  $P < 0.05$ .

## Results

Mean fetal weights of litters of normal and diabetic mothers are shown in Fig. 1. These results were obtained from the fetuses used in the subsequent experiments. The mean body weights of fetuses obtained from diabetic mothers on both 20.5 and 19.5 days of gestation were significantly greater than the mean body

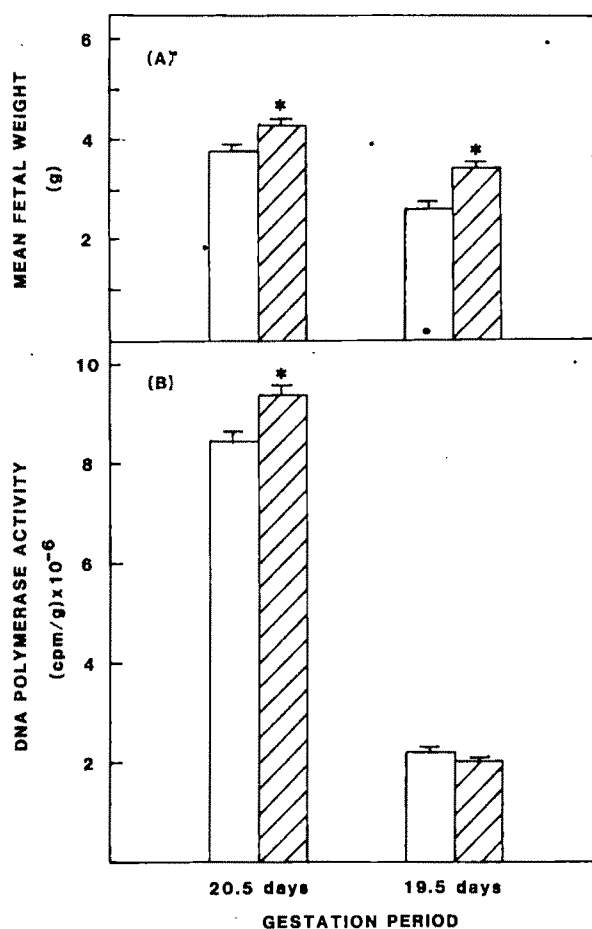


**Fig. 2.** Relationship between fetal weight and fetal DNA polymerase activity. *Panel A* shows mean values of average fetal weights from litters obtained from normal (open bars) and mildly diabetic (shaded bars) mothers on days 20.5 and 19.5 of gestation. The height of each bar shows the mean value (with standard error) of five average fetal weights of the litters in each group. *Panel B* shows the tissue level of DNA polymerase activities in fetuses shown in *Panel A*. Each bar represents the mean value (with standard error) from five litters in each group. Asterisks show  $P < 0.05$  when compared with corresponding normal groups.

weights of fetuses from corresponding normal mothers. These results indicated that macrosomic litters could be obtained as early as day 19.5 of gestation (2 days before birth).

Table I shows the rate of DNA synthesis and the rate of thymidine influx in normal and macrosomic fetuses. On day 20.5, the DNA content of the macrosomic fetus was significantly larger than that of the normal fetus, and the amounts of radioactive thymidine in both the total pool and the DNA pool of the macrosomic fetus were significantly greater than those in the corresponding pools of the normal fetus. Furthermore, the results



Table I. Effect of maternal diabetes on fetal thymidine pools

| Fetal status             | Fetal weight<br>(gm ± SEM) | DNA content<br>(mg/fetus ± SEM) | <sup>3</sup> H-thymidine in total pool<br>(cpm/fetus ± SEM) × 10 <sup>-3</sup> | <sup>3</sup> H-thymidine in DNA pool<br>(cpm/fetus ± SEM) × 10 <sup>-3</sup> |
|--------------------------|----------------------------|---------------------------------|--------------------------------------------------------------------------------|------------------------------------------------------------------------------|
| 20.5-day-old fetus from: |                            |                                 |                                                                                |                                                                              |
| Normal mother (n = 5)    | 4.27 ± 0.13                | 13.2 ± 0.5                      | 274 ± 6                                                                        | 84 ± 4                                                                       |
| Diabetic mother (n = 5)  | 4.79 ± 0.18                | 15.6 ± 0.7                      | 384 ± 7                                                                        | 159 ± 7                                                                      |
| t                        | P < 0.05                   | P < 0.05                        | P < 0.05                                                                       | P < 0.05                                                                     |
| 19.5-day-old fetus from: |                            |                                 |                                                                                |                                                                              |
| Normal mother (n = 5)    | 2.62 ± 0.13                | 8.02 ± 0.37                     | 119 ± 8                                                                        | 31 ± 3                                                                       |
| Diabetic mother (n = 5)  | 2.94 ± 0.07                | 9.30 ± 0.23                     | 224 ± 7                                                                        | 61 ± 2                                                                       |
| t                        | P < 0.05                   | P < 0.05                        | P < 0.05                                                                       | P < 0.05                                                                     |

Table II. Effect of maternal diabetes on fetal leucine pools

| Fetal status             | Fetal weight<br>(gm ± SEM) | Protein content<br>(mg/fetus ± SEM) | <sup>3</sup> H-leucine in total pool<br>(cpm/fetus ± SEM) × 10 <sup>-3</sup> | <sup>3</sup> H-leucine in protein pool<br>(cpm/fetus ± SEM) × 10 <sup>-3</sup> |
|--------------------------|----------------------------|-------------------------------------|------------------------------------------------------------------------------|--------------------------------------------------------------------------------|
| 20.5-day-old fetus from: |                            |                                     |                                                                              |                                                                                |
| Normal mother (n = 5)    | 4.28 ± 0.12                | 284 ± 5                             | 100.6 ± 18.0                                                                 | 3.6 ± 0.3                                                                      |
| Diabetic mother (n = 5)  | 4.81 ± 0.17                | 341 ± 7                             | 115.9 ± 17.1                                                                 | 4.9 ± 0.5                                                                      |
| t                        | P < 0.05                   | P < 0.05                            | P < 0.05                                                                     | P < 0.05                                                                       |
| 19.5-day-old fetus from: |                            |                                     |                                                                              |                                                                                |
| Normal mother (n = 5)    | 2.65 ± 0.13                | 184 ± 5                             | 55.0 ± 4.8                                                                   | 2.9 ± 0.2                                                                      |
| Diabetic mother (n = 5)  | 2.96 ± 0.10                | 203 ± 6                             | 68.8 ± 7.1                                                                   | 7.5 ± 0.6                                                                      |
| t                        | P < 0.05                   | P < 0.05                            | P < 0.05                                                                     | P < 0.05                                                                       |

Table III. Comparison between macrosomic fetus and normal littermates from diabetic mother

| Fetal status               | Fetal weight<br>(gm) | DNA content<br>(mg/fetus) | Polymerase activity<br>(cpm/gm) × 10 <sup>-6</sup> | <sup>3</sup> H-thymidine in total<br>pool (cpm/fetus) × 10 <sup>-3</sup> | <sup>3</sup> H-thymidine in DNA<br>pool (cpm/fetus) × 10 <sup>-3</sup> |
|----------------------------|----------------------|---------------------------|----------------------------------------------------|--------------------------------------------------------------------------|------------------------------------------------------------------------|
| Litter No. 1               |                      |                           |                                                    |                                                                          |                                                                        |
| Macrosomic fetuses (n = 1) | 3.02                 | 8.88                      | —                                                  | 1,167                                                                    | 442                                                                    |
| Normal fetuses (n = 10)    | 2.46                 | 7.70                      | —                                                  | 146                                                                      | 52                                                                     |
| Litter No. 2               |                      |                           |                                                    |                                                                          |                                                                        |
| Macrosomic fetuses (n = 3) | 2.93                 | 9.14                      | —                                                  | 700                                                                      | 353                                                                    |
| Normal fetuses (n = 9)     | 2.65                 | 8.11                      | —                                                  | 110                                                                      | 47                                                                     |
| Litter No. 3               |                      |                           |                                                    |                                                                          |                                                                        |
| Macrosomic fetuses (n = 1) | 3.12                 | 10.05                     | 2.12                                               | —                                                                        | —                                                                      |
| Normal fetuses (n = 10)    | 2.77                 | 8.25                      | 2.21                                               | —                                                                        | —                                                                      |
| Litter No. 4               |                      |                           |                                                    |                                                                          |                                                                        |
| Macrosomic fetuses (n = 2) | 2.96                 | 9.22                      | 2.30                                               | —                                                                        | —                                                                      |
| Normal fetuses (n = 8)     | 2.54                 | 7.92                      | 2.26                                               | —                                                                        | —                                                                      |

from the 19.5-day fetuses were very similar to those of the 20.5-day fetuses. The stimulation of DNA synthesis as well as thymidine influx in macrosomic fetuses occurred as early as day 19.5 of gestation.

Fig. 2 depicts the relationship between fetal macrosomia and fetal DNA polymerase activities. The fetuses obtained on day 20.5 showed that the tissue levels of DNA polymerase activities in macrosomic fetuses were significantly higher than the tissue activities of the enzyme in normal fetuses. On the other hand, tissue levels of enzyme in macrosomic fetuses obtained on day 19.5 were not significantly different

from the activities in normal fetuses. In contrast to the 20.5-day fetuses, macrosomia in the 19.5-day fetuses did not accompany stimulation of DNA polymerase activity.

Table II shows the rate of protein synthesis and the rate of amino acid influx in normal and macrosomic fetuses. The mean fetal weights and total protein contents indicated that fetuses obtained from diabetic mothers on both day 20.5 and day 19.5 were macrosomic. The rate of leucine transport into fetuses as measured by the amount of total <sup>3</sup>H-leucine incorporation was significantly greater in macrosomic fetuses

than in normal fetuses. The rate of protein synthesis as measured by the amount of  $^3\text{H}$ -leucine incorporation into protein was greater in macrosomic fetuses than in normal fetuses. Increase in both the protein synthesis and transport of the precursor occurred in macrosomic fetuses as early as day 19.5 of gestation.

The macrosomic fetuses and normal littermates from diabetic mothers are compared in Table III. All of these litters were obtained on day 19.5 of gestation. Litters No. 1 and No. 2 showed that DNA synthesis and transport of thymidine were greater in the macrosomic fetuses than in their normal littermates. Litters No. 3 and No. 4 showed that DNA polymerase activities in the macrosomic fetuses were not significantly different from the enzyme activities in their normal littermates. The results were essentially the same as those shown in Fig. 2 and Table I.

#### Comment

Under different pathophysiologic conditions in a variety of mammalian tissues, changes in tissue mass have been correlated with concomitant changes in the rate of DNA synthesis, and the changes in DNA synthesis have been attributed to the tissue levels of DNA polymerase activities.<sup>14-18</sup> Our earlier observation<sup>5</sup> indicated that neonatal macrosomia accompanies increase in the DNA content and stimulation of DNA polymerase activities, but whether or not stimulation of this enzyme initiates the fetal macrosomia has not been shown. The present study shows that manifestation of fetal macrosomia precedes a noticeable increase in the tissue level of the enzyme activity (Fig. 2), but the stimulation of the polymerase occurs only subsequently in macrosomic fetuses. These results were interpreted as meaning that stimulation of DNA polymerase- $\alpha$  participates in the maintenance of macrosomia but is not an initiating cause of macrosomia. The DNA polymerase data for littermates (Table III) also provide strong evidence that stimulation of DNA polymerase is not an antecedent condition for manifestation of macrosomia.

Increases in the nitrogen retention and increases in the contents of DNA and proteins in macrosomic fetuses and neonates have been shown previously.<sup>5, 19</sup> However, whether or not the rate of synthesis of these macromolecules is stimulated in macrosomic fetuses and neonates and, if so, the mechanism of stimulation have not been elucidated. The results reported here show that the rates of synthesis of both DNA and proteins are increased in macrosomic fetuses as early as day 19.5 of gestation (Tables I and II). Furthermore, the rates of transport of the precursors from the ma-

ternal side to the fetal side are considerably increased in the macrosomic litters (Tables I and II).

The increase in synthesis of DNA and proteins reported here is consistent with the increase in the tissue content of these macromolecules in the macrosomic fetuses. Nevertheless, the possibility of a decrease in the rate of degradation as an additional mechanism for fetal macrosomia cannot be excluded on the basis of present data.

The pulse-labeling method has been widely used for estimating rates of synthesis of macromolecules and rates of precursor influx. However, results from such experiments require a careful analysis. Plasma levels of amino acids and carbohydrates (leucine, glutamine, and glucose) in both fetal and maternal sides have been shown to be higher in maternal diabetes.<sup>20, 21</sup> Therefore, in the experiments reported here, the specific radioactivities of leucine in the maternal circulation and in the fetal circulation should be lower in the diabetic mother and its fetus than in the normal mother and fetus. The plasma levels of thymidine in diabetic rats and their fetuses are not known. However, the experiments with littermates (Table III) provide the evidence for an increase in the rate of transport of thymidine and a corresponding increase in DNA synthesis in macrosomic fetuses. Since the fetal source of thymidine depends on synthesis from amino acids and carbohydrates in the fetus, the specific radioactivity of the thymidine precursor pool in the macrosomic fetus (in the diabetic mother) should be lower than that in the normal fetus (of the normal mother). These observations suggest that the stimulation of transport of precursors and synthesis of the macromolecules in the macrosomic fetus would in reality be greater than that indicated by the radioactivity data presented here.

Finally, exact mechanisms underlying the stimulation of synthesis of DNA and proteins in the macrosomic fetus are not clearly understood. However, increased substrate levels (i.e., increase in precursor pool sizes) offer a possible mechanism by which the synthetic activities are stimulated. Increase in transport of metabolites from the maternal side to the fetal side appears to be one of the early changes associated with development of macrosomia in diabetic pregnancy. This proposal is consonant with reports showing structural and functional alterations in vascular systems induced by diabetes.<sup>22-24</sup> Furthermore, it has long been observed that pregnancy complicated by maternal diabetes induces fetal hyperglycemia and hyperinsulinemia,<sup>7, 25</sup> and it has long been known also that insulin promotes cellular transport of amino acids and subsequent synthesis of proteins.<sup>26, 27</sup> In fact, fetal hy-



perinsulinemia has been implicated in stimulation of fetal and placental growth.<sup>28, 29</sup> Nevertheless, the actual temporal sequence of increases in the glucose and insulin levels and increases in the substrate influx into macrosomic fetuses has yet to be correlated.

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# Short-term variability of fetal heart rate during pregnancies with normal and insufficient placental function

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The differential index (DI) describing the short-term component of the fetal heart rate (FHR) was measured serially in 65 fetuses during normal pregnancies, in 68 fetuses with suspected intrauterine growth retardation (IUGR), and in 84 fetuses with suspected postmaturity. The analyses of FHR variability were made by a microprocessor-based "on-line" method with the use of the abdominal fetal electrocardiogram (aFECG) in the statistical calculation of the interval differences. The analysis of FHR variability was successful 463 of 581 times (80%) in the reference group, 73 of 89 times (82%) in the group with suspected IUGR, and 121 of 127 times (95%) in the group with suspected postmaturity. There was no fetal distress in the reference group, but fetal distress did occur in 24 (35%) of the IUGR fetuses and in 13 (15%) of the postmature fetuses. The first percentile limit of DIs in the normal group was used as the normal limit of DI for fetuses with IUGR. In the postmature group the cutoff level of DI was set at 3.5. The sensitivity of DI in predicting fetal distress was 64% in the group with suspected IUGR and 54% in the group with suspected postmaturity. The predictive values of DI were 80% and 58%, respectively. The risk ratios of abnormal and normal DIs were 3.6 in suspected IUGR and 6.8 in suspected postmaturity. Hence, DI seems to be a sensitive and reliable indicator of developing fetal distress in suspected IUGR and postmaturity. The fairly high failure rate of aFECG at the onset of the third trimester of pregnancy limits the practical value of FHR analysis. (AM. J. OBSTET. GYNECOL. 139:33, 1981.)

ONE OF THE MOST DIFFICULT problems in modern obstetrics is to detect the true cases of suspected intrauterine growth retardation (IUGR) and postmaturity. The main reason for this dilemma is the unknown time of ovulation. However, we know that uteroplacental insufficiency (UPI), which is the cause of fetal distress in both of these risk groups, may conflict with the course of pregnancy at any time. We also know that at the end point of UPI the human fetus consistently shows late decelerations and a fixed baseline of the fetal heart rate (FHR). Observation of late decelerations with spontaneous uterine contractions would require

impractically long recordings of FHR in all cases of suspected UPI. A consistent fixed baseline of FHR, on the other hand, may be too late as a sign of fetal jeopardy because the fetal brain may have been damaged when the sign is manifest.

This investigation was based on the hypothesis that the reduction of FHR variability can be detected by automated statistical methods before it is visually recognizable in the cardiotocogram. With this method, the time-consuming task of detecting true IUGR and postmaturity could be avoided and the obstetrician could concentrate on identifying fetuses with chronic hypoxia caused by placental degeneration.

Methods for quantifying FHR variability during labor from a direct fetal electrocardiogram (FECG) have been presented by several research groups.<sup>1-6</sup> Only two groups have published results from the antepartum period on a quantitative basis.<sup>4, 7</sup> In this report the differential index (DI) introduced by Yeh and associates<sup>8</sup> was used as a measure of the short-term variability of FHR.

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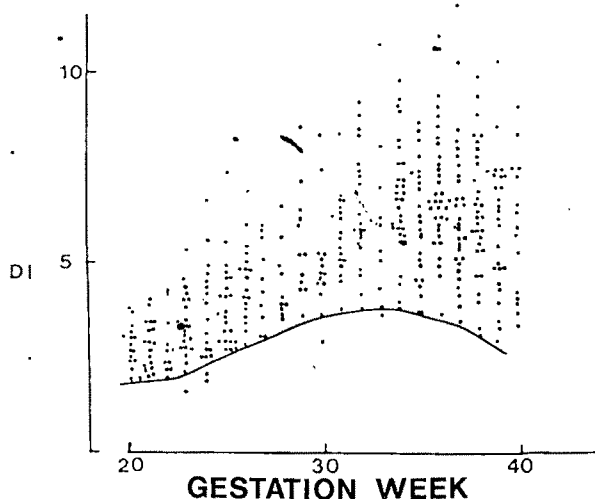


Fig. 1. Scattergram of DIs measured from 5-minute samples of abdominal fetal electrocardiograms during 65 normal pregnancies. The lower first percentile limit is drawn as the normal limit of DI.

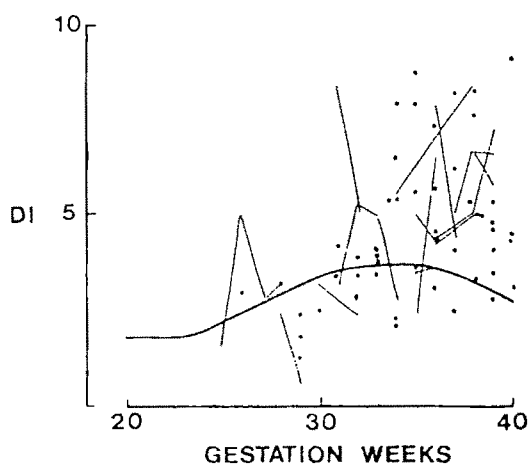


Fig. 2. Development of DIs measured from 5-minute samples of abdominal fetal electrocardiograms of 61 fetuses with suspected intrauterine growth retardation. The normal limit of DI from Fig. 1 is drawn for reference.

#### Patients and methods

**Normal pregnancies.** The reference material was collected from uncomplicated singleton pregnancies of private patients whose fetuses experienced a good outcome. There were no maternal diseases except fetopelvic disproportion. All fetuses were monitored by cardiotocography during labor or before cesarean section. There were no late decelerations or fixed baseline of FHR. All had an Apgar score of more than 7. None had acidosis during labor. A total of 463 DIs were measured at 1- to 4-week intervals in these 65 fetuses.

**IUGR.** This group consisted of 68 pregnant patients

referred from the maternity outpatient clinics to the Departments of Obstetrics and Gynecology, Helsinki University Central Hospital. IUGR was suspected when the symphysis-fundus distance or biparietal diameter fell below the fifth percentile of the normal population. The weights of the newborn infants ranged from 585 to 3,630 gm (mean 2,043 gm  $\pm$  814 SD). Thirty-five were delivered by cesarean section. Five perinatal deaths occurred in this group: One infant had a severe fetomaternal transfusion; one infant had a congenital malformation of the kidneys; one infant had trisomy-18; one infant had a diabetic mother (White Class F), and one infant died of respiratory distress syndrome and intracerebral hemorrhage. Eleven babies had Apgar scores of less than 7 at 1 or 5 minutes. Twenty-four fetuses showed late decelerations and 26 had a fixed baseline of FHR during labor or before cesarean section. A fetal blood analysis was performed in two; neither had acidosis. Twenty-five fetuses had two or more signs of fetal distress. One of the survivors had trisomy-21. A total of 73 DIs were measured in this group.

**Postmaturity.** A total of 84 pregnant patients in this group were sent to our clinic for suspected postmaturity. Postmaturity was suspected when the pregnancies were 10 days or more past term according to the 28-day period. No perinatal deaths occurred in this group. Ten babies were delivered by cesarean section and nine were delivered by vacuum extraction; the rest were delivered spontaneously. Four had Apgar scores less than 7, and six had signs of acidosis following fetal blood analysis. Thirty-three fetuses showed late decelerations and 20 had a fixed baseline of FHR. Thirteen fetuses had two or more signs of fetal distress. A total of 121 DIs were measured in this group.

**Fetal distress.** Included in the category of fetal distress were perinatal death or the occurrence of two of the following symptoms: more than two late decelerations during labor or before cesarean section, fixed baseline of FHR (total variability less than 5 bpm by visual inspection) for more than 5 minutes in the same circumstances, actual pH less than 7.20 or base excess less than  $-13$  (Saling method), and Apgar score less than 7 at 1 or 5 minutes after birth.

**Measurement of the short-term variability of FHR.** DIs were measured by a previously described method.<sup>9</sup> The sample time of 5 minutes was chosen because DI is insensitive to the sample time<sup>3</sup> and the short recording time allowed the test to be performed even in a supine position. The upper rejection limit for beat-to-beat differences was 5 bpm. Two silver cup electrodes with recording paste and an HP-8030A cardiotocograph were used for recording the abdominal FECCG.

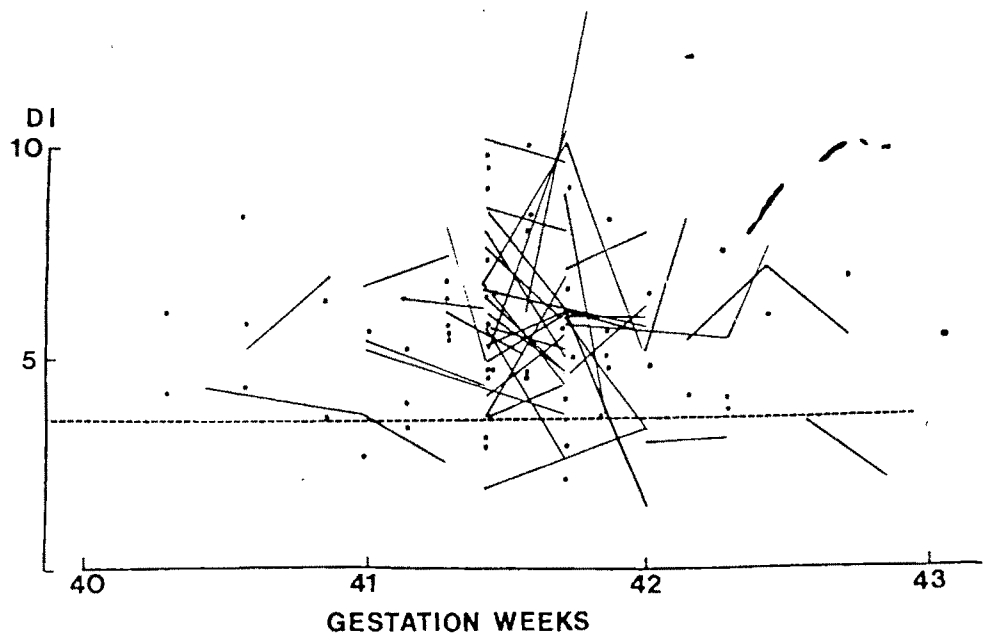


Fig. 3. Development of DIs measured from 5-minute samples of abdominal fetal electrocardiograms of 82 postterm fetuses. The dotted line indicates an arbitrarily chosen cutoff level of DI. Gestational days have been calculated according to the individual lengths of periods.

Table I. Results of the FHR analyses performed in 152 fetuses with suspected placental insufficiency

| Risk group             | No. of fetuses | Failures of analysis | DI       | Fetal distress |    | Sensitivity (%) | Predictive value (%) | Relative risk | p        |
|------------------------|----------------|----------------------|----------|----------------|----|-----------------|----------------------|---------------|----------|
|                        |                |                      |          | Yes            | No |                 |                      |               |          |
| Suspected IUGR         | 68             | 7                    | Abnormal | 16             | 4  | 64              | 80                   | 3.6           | < 0.01   |
|                        |                |                      | Normal   | 9              | 32 |                 |                      |               |          |
| Suspected postmaturity | 84             | 2                    | Abnormal | 7              | 5  | 54              | 58                   | 6.8           | < 0.002  |
|                        |                |                      | Normal   | 6              | 64 |                 |                      |               |          |
| Total                  | 152            | 9                    | Abnormal | 23             | 9  | 61              | 72                   | 5.5           | < 0.0001 |
|                        |                |                      | Normal   | 15             | 96 |                 |                      |               |          |

The normal limit of DI in the group with suspected IUGR is explained in Fig. 1. The abnormal values of DI in the group with suspected postmaturity are less than 3.5. Statistical significance was assessed by the chi-square test from frequencies of which the relative risk was calculated.

The mothers lay supine or slightly tilted laterally. The analysis was regarded as a failure when less than 30% of fetal intervals were accepted in the analysis.

**Assessment of the test.** The results were divided into five categories: failure of analysis, correct positive results (fetuses with one or more abnormal DI and fetal distress), false positive results (fetuses with one or more abnormal DI without two signs of fetal distress), correct negative results (fetuses with normal DIs with no fetal distress), and false negative results (fetuses with normal DIs but fetal distress).

Three statistical descriptors were used to assess the clinical value of the test (DI): sensitivity, predictive value, and relative risk.<sup>10</sup> Sensitivity was defined by the percentage of fetuses with a correct positive result in the group with fetal distress. Predictive value was the

correct positive results as a percentage of all the positive results. The relative risk was the ratio of the percentage of true positive results to the percentage of false negative results. Statistical significance between the two percentages in the calculation of the relative risk was assessed by the chi-square test.

Results

**Normal pregnancies.** The distribution of the DIs as a function of normal gestation is presented in Fig. 1. Only 1% of the results fell below the limit drawn for normal. The DI tended to increase with advancing gestation. The DIs were not normally distributed.

**Suspected IUGR.** The distribution of the DIs in suspected IUGR can be seen in Fig. 2. The difference between the distributions in Figs. 1 and 2 is highly sig-



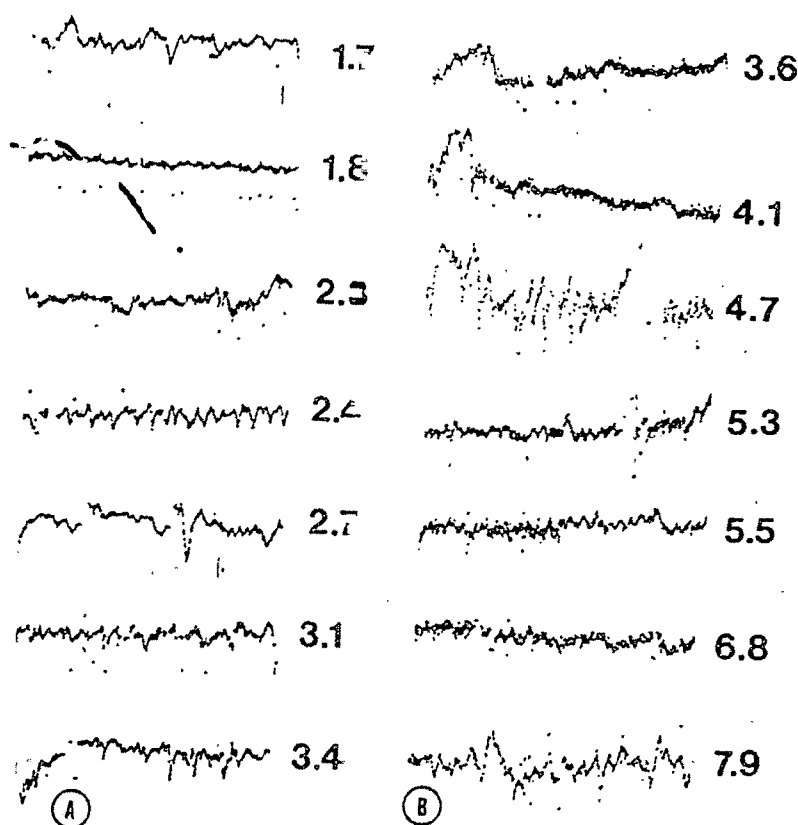


Fig. 4. Six true positive results (A) and six true negative results (B) of fetal heart rate analyses (DIs) performed for fetuses with suspected IUGR and the corresponding strips of FHR tracings.

nificant ( $p < 0.001$ ,  $\chi^2$  test) Twenty of 68 fetuses had abnormal DIs. Four of these were false positive. Nine fetuses had normal DIs although they developed fetal distress (false negative) (Table I). FHR analysis of seven fetuses failed.

**Suspected postmaturity.** The distribution of the DIs according to the gestational day is presented in Fig. 3. No significant trend in the DI was observed during the postdate period. Twelve of 84 fetuses had abnormal DIs. Five of these were false positive. Six fetuses had false negative DIs (Table I). FHR analysis of two fetuses failed.

**Assessment of DI as a test for fetal distress.** Sensitivity, predictive value, and relative risk of DI as a test for developing fetal distress are given in Table I, which also shows the frequency of failure of FHR analysis in the various groups. The relative risks of abnormal and normal DIs in both groups and in the whole series are highly significant.

#### Comment

The development of FHR variability during normal gestation has been described previously on a semi-quantitative<sup>11</sup> and a quantitative basis.<sup>7, 9</sup> This study

confirms the rising trend of beat-to-beat variability during normal gestation. Limits for normal values have not previously been set. The cutoff level of the first percentile chosen by us is natural for the DI distribution. The significantly different distribution of DIs in the normal group and in the group of suspected IUGR supports the selection of this limit. The cutoff level of 3.5 for the postterm fetuses is arbitrary, but the distribution of the DIs in the postterm group suggests that this limit also is meaningful.

FHR analysis was almost always successful in the group with suspected postmaturity. Analysis very probably would have succeeded in the two unsuccessful cases if the fetus had not moved during the recording. This finding suggests that the abdominal FECG should always be used when a cardiotocogram is recorded after term. This would help to avoid misinterpretation of FHR variability caused by trigger error in the ultrasound. The fairly high failure rate of FHR analysis in the normal and IUGR groups is attributable to the failure of the abdominal FECG at the onset of the third trimester of pregnancy. This concurs with earlier studies on the signal size of the abdominal FECG.<sup>12</sup> The reasons for this phenomenon are not known.

The risk groups of pregnancies studied here—suspected IUGR and postmaturity—are usually associated with insufficient placental function. Perinatal death is very uncommon among postterm pregnancies (none in 1979 in our clinic with over 5,700 deliveries a year) and rare in the whole population (7.8/1,000), and hence it cannot be used as a sole criterion of fetal distress. Therefore, the most reliable signs of fetal hypoxia due to UPI, late decelerations and a fixed baseline of FHR, were selected as indicating fetal distress. Since they were fairly common in the group of suspected IUGR, two additional signs, fetal acidosis and a low Apgar score, were applied. The presence of two of these four would very probably indicate insufficient placental function.

The clinical decisions to treat the fetuses in the risk groups were based mainly on the nonstress test of cardiotocography. The oxytocin challenge test is not used in our clinic. The results of the FHR analysis did not influence the decisions. The fairly good fetal outcome (perinatal death rate of 3.3%) in these high-risk groups is obviously related to the experience of the obstetricians in interpreting cardiotocograms and to intensive neonatal care. Comparison of the FHR analysis with nonstress cardiotocography was not the aim of this study, but some such comparison is evidently needed.

The DI was more sensitive and more specific in detecting fetal distress in the group with suspected IUGR

than in the group with suspected postmaturity. On the other hand, the relative risk was higher in the group of postterm fetuses, probably because of the low risk of fetal distress after a normal DI.

Could these results have been obtained by visual inspection of the cardiotocograms by experienced obstetricians? It has been shown that visual evaluation of FHR variability is problematic, but its accuracy can be improved by training.<sup>13</sup> It is evident from a comparison of the true positive results of DI in the suspected IUGR group of this study with some of the true negative results (Fig. 4) that it would be extremely difficult to evaluate these recordings visually.

The main advantage of FHR analysis versus biochemical antepartum fetal monitoring is that the results of the analysis are available immediately. The short recording time compared with that of nonstress cardiotocography will make it more practical as a screening tool. The main disadvantage of FHR analysis is the fairly high failure rate of the abdominal FECG at the onset of the third trimester of pregnancy. According to this study, DI seems to be a sensitive and reliable indicator of developing fetal distress in postterm and growth-retarded fetuses.

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# Maternal and cord blood complement activity: Relationship to premature rupture of the membranes

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Human complement levels are known to reflect the presence of clinical infection, with low levels indicative of increased utilization in the body's immune response. Premature rupture of the membranes (PRM) is associated with an increased maternal-fetal infectious morbidity, yet histopathologic and bacteriologic parameters often do not reflect this. In this prospective study maternal and cord serum total hemolytic complement activity ( $CH_{50}$ ) was measured in 102 patients without evidence of infection, with and without PRM, to determine if there exists within the maternal-fetal unit an immunologic response to this event. It was found that maternal serum  $CH_{50}$ , regardless of length of gestation, is similar to that of the nonpregnant adult population, except in cases of PRM greater than 12 hours in which it is significantly lower. The  $CH_{50}$  in term newborn infants was significantly lower than that of matched maternal samples and dropped with decreasing gestational age.  $CH_{50}$  in cord serum from babies of mothers with PRM failed to show a significant decrease when compared to those of mothers without PRM. (AM. J. OBSTET. GYNECOL. 139:38, 1981.)

MANAGEMENT DECISIONS with regard to patients with premature rupture of the membranes (PRM) are mainly based on findings of maternal-fetal infection. Although histopathologic and bacteriologic documentation suggesting infection points to PRM as a major predisposing factor,<sup>1, 2</sup> significant clinical maternal-fetal infection in such cases is often absent.<sup>3</sup> Human complement levels are known to reflect the presence of clinical infection with low values indicative of increased utilization in the immune response.<sup>4, 5</sup> Measurement of the various components (C3, C5, etc.) of the complement system is a quantitative one done at a certain point in the cascade of activity. Total hemolytic complement activity ( $CH_{50}$ ) is a functional measurement and more accurately reflects the sum degree of complement fixation.<sup>6</sup> In this prospective study we examined maternal and cord  $CH_{50}$  to determine if there is an immune response within the maternal-fetal unit trig-

gered by PRM, possibly indicating infection prior to its becoming clinically evident.

## Subjects and methods

Obstetric inpatients (including maternal transfers) from Ochsner Foundation Hospital and their infants made up the study group from which we took 102 maternal and 102 matched cord blood samples. Any patient with clinical evidence of intrapartum chorioamnionitis or endometritis (fever-tachycardia, leukocytosis, uterine tenderness) was excluded from the study. Maternal blood samples were taken within 2 hours before delivery from three groups of 34 women: (1) patients at term (37 weeks or greater); (2) patients at less than 37 weeks' gestation; and (3) patients with rupture of the membranes earlier than 12 hours before delivery. In this group 15 had ruptured membranes 12 to 24 hours of duration, 15 had ruptured membranes 24 to 48 hours of duration, and three patients had ruptured membranes greater than 48 hours.

Cord serum samples were grouped as follows: (1) term, (2) less than 37 weeks, (3a) term, with PRM more than 12 hours, and (3b) preterm, with PRM more than 12 hours. All postpartum women and newborn infants were evaluated for infection by clinical measures and appropriate cultures where indicated.

We measured complement activity by the  $CH_{50}$  technique, with the use of an initial dose of 0.3 ml of

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Table I. Maternal serum CH<sub>50</sub>

| Group             | n  | Range  | Mean CH <sub>50</sub> ± SD | Significance*   |
|-------------------|----|--------|----------------------------|-----------------|
| 1. ≥ 37 weeks     | 34 | 25-141 | 91.20 ± 25.82              | NS<br>p < 0.001 |
| 2. < 37 weeks     | 34 | 30-138 | 80.97 ± 29.62              |                 |
| 3. PRM > 12 hours | 34 | 10-141 | 65.02 ± 30.42              |                 |

CH<sub>50</sub> = Total hemolytic complement activity (percentage of normal adult value) SD = 1 standard deviation from mean. NS = Not significant.

\*Student's t test.

Table II. Cord serum CH<sub>50</sub>

| Group                             | n  | Range | Mean CH <sub>50</sub> ± SD | Significance*                |
|-----------------------------------|----|-------|----------------------------|------------------------------|
| 1. ≥ 37 weeks                     | 34 | 5-154 | 62.44 ± 34.91              | p < 0.001<br>p < 0.001<br>NS |
| 2. < 37 weeks                     | 34 | 2-102 | 31.87 ± 23.51              |                              |
| 3a. ≥ 37 weeks,<br>PRM > 12 hours | 15 | 17-86 | 45.80 ± 33.14              |                              |
| 3b. < 37 weeks,<br>PRM > 12 hours | 19 | 12-75 | 31.20 ± 25.12              |                              |

CH<sub>50</sub> = Total hemolytic complement activity (percent of normal adult value). SD = 1 standard deviation from mean. NS = Not significant.

\*Student's t test.

serum, a progressive dilution system of 8/10, and calculation according to a new, more precise equation.<sup>6, 7</sup> The final dilution volume in each tube was 0.6 ml, to which 0.6 ml of sensitized corpuscles adjusted to an optical density of 1.4 was added. Under these conditions, the normal adult level is 100 U. However, because of the large variations in unit size reported in the literature, values are reported here in percentages of normal adult values.

Results

**Maternal samples.** The distribution of CH<sub>50</sub> values from the maternal serum is shown in Table I. The relatively large standard deviations seen are common in measurements of CH<sub>50</sub> by this technique.<sup>7</sup> Values for term and preterm patients were similar. In those women delivered more than 12 hours after rupture of the membranes, the mean CH<sub>50</sub> (65.20) was significantly lower than that of both groups without PRM (91.20 and 80.97).

There were only two cases of postpartum endometritis, both from the PRM group. The predelivery CH<sub>50</sub> values in these two patients were 30 and 59.2.

**Cord samples.** Table II shows the distribution of complement values in the cord serum. Differences by gestational age (Group 1 versus Group 2 and Group 3a versus 3b) were significant, but differences between the groups with and without PRM were not significant. Term newborn infants of mothers with PRM (Group 3a), when compared with those without PRM (Group 1), demonstrated an apparent decrease in mean CH<sub>50</sub>, but the difference was not significant. One term new-

born infant, whose mother had PRM, subsequently grew Group B β-hemolytic streptococci from a surface culture. The baby had no clinical evidence of infection and had a cord CH<sub>50</sub> of 85. Five preterm infants from Group 3b were clinically suspected of having an infection, including leukocytosis, but none had positive surface or blood cultures.

Comment

The complement system, a component of the body's immune mechanism, is composed of a series of inactive proteins circulating in the extracellular fluid compartment. In the presence of infection, the system is activated through either the classical (binding of antibody molecule) or properdin pathway and cascades toward production of active intermediaries in the immune response.<sup>4</sup> Levels of complement then fall below normal values, indicating that complement is being consumed in the body's defense.<sup>4, 5</sup>

Complement activity levels in pregnancy have been shown to be similar to those of nonpregnant women.<sup>8</sup> Our results showing a significantly lower mean CH<sub>50</sub> in those patients with PRM greater than 12 hours are the first reported evidence of what appears to be an early maternal immunologic response elicited by ruptured membranes. The CH<sub>50</sub> decrease may result from the release of fluid containing antigens heterologous to the mother, triggering an antigen-antibody reaction with consumption of complement, and/or from the presence of infective agents that initiate the complement reaction as a protective mechanism. Only two patients (from the PRM group) had postpartum endometritis



and the  $CH_{50}$  levels before delivery were both below the mean for the group. It is conjectured at this point to state that this signifies a response to subclinical maternal infection. Further prospective studies of patients with proved infection may clarify this association and suggest a clinically useful parameter.

Ontogeny of complement synthesis begins as early as 10 weeks of fetal age<sup>9</sup> from a yet undetermined source (although peritoneal and alveolar cells from the 14-week fetus produced complement *in vitro*)<sup>10</sup> and precedes ontogeny of immunoglobulin synthesis.<sup>11</sup> Complement does not cross the placenta<sup>12, 13</sup>; therefore, cord serum reflects newborn  $CH_{50}$  exclusively.

Complement activity in the term fetus has been shown to be approximately half of matched maternal levels.<sup>9, 11, 13, 14</sup> Normal adult levels are reached by 6 months of age.<sup>11, 15</sup> In premature infants, complement levels tend to be lower with decreasing gestational age.<sup>11, 13, 16</sup> Our results support these findings. The relative state of complement deficiency in both term and preterm newborn infants raises the possibility of impaired complement-dependent immunologic function and may partially explain the incidence of serious infection, especially in preterm neonates,<sup>13, 17, 18</sup> who in our study showed no response to PRM.

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# Protein synthesis by human fetal lung

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Biochemical and morphologic studies have shown that proteins and carbohydrate-protein complexes, in addition to phospholipids, are present in the alveolar surface layer. For this reason the study of lung protein synthesis in parallel with phospholipid biosynthesis and its regulation is of interest. The data obtained show that from 16 to 24 weeks' of gestation the human fetal lung rapidly incorporated  $^{14}\text{C}$ -leucine into proteins from the particulate fraction and  $105,000 \times \text{g}$  tissue supernatant fraction. The distribution of radioactivity between protein fractions after gel filtration on Sephadex G-200 revealed three major peaks of radioactivity. According to our results the protein, detected at the site of the 7S peak of normal serum, was synthesized in vitro by human lung tissue, as was a more rapidly labeled 19S protein which was released into the incubation medium. The addition of prolactin and cortisone to the incubation medium led to a dose-dependent inhibition of total protein synthesis by the human fetal lung in vitro. The significant decrease in  $^{14}\text{C}$ -leucine incorporation was noticed in proteins eluted from the Sephadex G-200 column in fractions corresponding to the 19S and 7S peaks of normal human sera. (AM. J. OBSTET. GYNECOL. 139:41, 1981.)

FETAL, MATERNAL, and amniotic fluid hormones undoubtedly influence fetal pulmonary growth and development as well as that of other organs. Pulmonary immaturity, which leads to the respiratory distress syndrome (RDS), is caused by inability of the type II pneumocytes of the fetal lungs<sup>1, 2</sup> to synthesize adequate quantities of surfactant.

Present evidence indicates that thyroxine,<sup>3-6</sup> cortisone,<sup>7-10</sup> and prolactin<sup>12</sup> play an important role in the development of the lung in utero. The influence of growth hormone, estradiol, and testosterone has not been fully defined.

The major surface-active component of surfactant is a specific lecithin, dipalmitoyl phosphatidylcholine, also called dipalmitoyl lecithin. Biochemical and morphologic studies have shown that proteins and car-

bohydrate-protein complexes are also present in the alveolar surface layer. For this reason the study of lung protein synthesis in parallel with phospholipid biosynthesis and its regulation is of interest.

The evidence that mature lung tissue can synthesize proteins rapidly in vivo and in vitro came from Massaro and associates.<sup>13</sup> The absence of information concerning protein synthesis by the human fetal lung and its regulation led to the present work, which showed that human fetal lungs synthesize proteins actively and that prolactin and cortisone inhibit the rate of in vitro incorporation of  $^{14}\text{C}$ -leucine into such proteins.

## Material and methods

**Clinical material.** Fresh lung tissue from one 16- to 18-week-old fetus and six fetuses of 22 to 24 weeks of age was obtained after cesarean sections performed for gynecologic-social indications on patients with mental retardation. Immediately after removal the tissue was placed at 4° C in a 0.9% sodium chloride solution until use; incubations were started within 2 hours.

**Incubation of fetal lung tissue.** Tissue fragments were minced in saline, with a wet weight averaging 0.5

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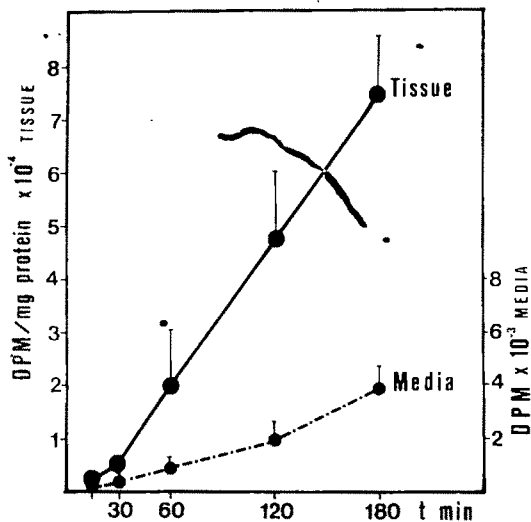


Fig. 1. The incorporation of  $^{14}\text{C}$ -leucine into TCA-precipitable proteins from  $12,000 \times g$  tissue supernatant and incubation medium. Each value is the mean of five independent experiments performed in duplicate.

gm, and were randomly placed in 10 ml Erlenmeyer flasks with 3 ml of Eagle's medium. Incubations were started by adding  $2.5 \mu\text{Ci}$  of uniformly labeled  $^{14}\text{C}$ -leucine (Batch 155, specific activity  $344 \text{ mCi/mmol}$ , The Radiochemical Centre, Amersham/Searle, England) and were continued for periods of 15, 30, 60, and 180 minutes in a Gallenkamp shaking water bath (50 cycles/min) under an atmosphere of 95% oxygen and 5% carbon dioxide. Duplicate incubations were used at each time point.

To determine the possible effect of amniotic fluid hormones on protein synthesis in the human fetal lung, the following hormones were added to the incubation medium: human prolactin (standard human prolactin preparation supplied by IRE France), insulin (Galenika, Yugoslavia), human placental lactogen (HPL) (lot No. 6033, ICM-Pharmaceutical), prostaglandin  $\text{F}_{2\alpha}$  ( $\text{PGF}_{2\alpha}$ ) (supplied by Dr. J. E. Pike, The Upjohn Co.), and cortisone.

**Processing of tissue and incubation media.** The incubation was terminated by immersing the Erlenmeyer flasks into an ice bath. Following incubation, the media and tissue fragments were filtered through two layers of cheesecloth; the filtrate was centrifuged at  $2,000 \times g$  for 10 minutes, the pellet was discarded, and the supernatant fraction was recentrifuged at  $10,000 \times g$  and kept frozen at  $-30^\circ \text{C}$  until use.

The tissue was removed from the cheesecloth and homogenized with a Teflon-coated pestle for 3 minutes in a 10 ml grinding vessel immersed in ice, saline or

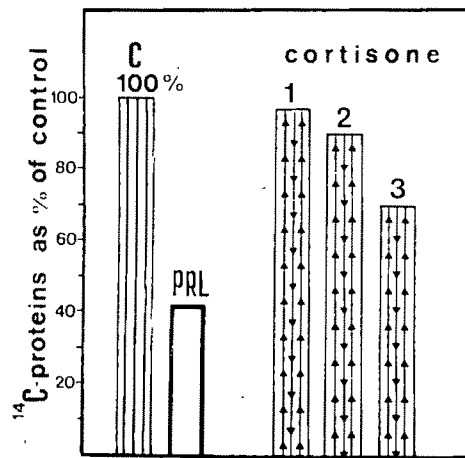


Fig. 2.  $^{14}\text{C}$ -proteins from  $105,000 \times g$  tissue supernatant after incubation of 18 week-old human fetal lung fragments treated with prolactin and cortisone. The results are expressed as a percentage of control. C = Control. PRL =  $4 \text{ mIU/ml}$ . Cortisone: 1,  $0.4 \text{ mg/ml}$ ; 2,  $0.8 \text{ mg/ml}$ ; 3,  $1.6 \text{ mg/ml}$ . Each value is the mean of three determinations.

$0.1\text{M}$  ammonium bicarbonate buffer, pH 8.5, was used. The crude homogenate was centrifuged at  $2,000 \times g$  for 15 minutes; the pellet was discarded, and the supernatant fraction was recentrifuged at  $10,000 \times g$  for 15 minutes. The  $10,000 \times g$  pellet was resuspended in 1 ml of Protosol (MEF 935 New England Nuclear Corp.) and assayed for radioactivity. The supernatant fraction was frozen at  $-30^\circ \text{C}$  until assayed or centrifuged for 1 hour at  $105,000 \times g$ , and the resulting supernatant fraction was collected and kept frozen until assayed.

**Determination of  $^{14}\text{C}$ -proteins.** Aliquots of  $0.1 \text{ ml}$  of incubation medium and  $10,000 \times g$  tissue supernatant fraction were precipitated with 10% cold trichloroacetic acid (TCA), filtered, and washed twice with 2 ml of 5% TCA through Sartorius membrane filters (SM 11406). The radioactivity was counted in Bray's solution in a liquid-scintillation counter (Model S1 4000, Inter-technique).

**Total protein determination.** The method described by Lowry and associates<sup>14</sup> was used for total protein determination. Highly purified bovine serum albumin was used as a standard.

**Gel filtration.** The  $105,000 \times g$  supernatant fraction of control and prolactin-treated lung tissue, which had been incubated for 3 hours with labeled leucine, was applied independently to a column of Sephadex G-100 ( $2.5 \text{ by } 65 \text{ cm}$ ) and G-200 ( $1 \text{ by } 70 \text{ cm}$ ). The columns were equilibrated and eluted with  $0.1\text{M}$   $\text{NH}_4\text{HCO}_3$  buffer, pH 8.3.

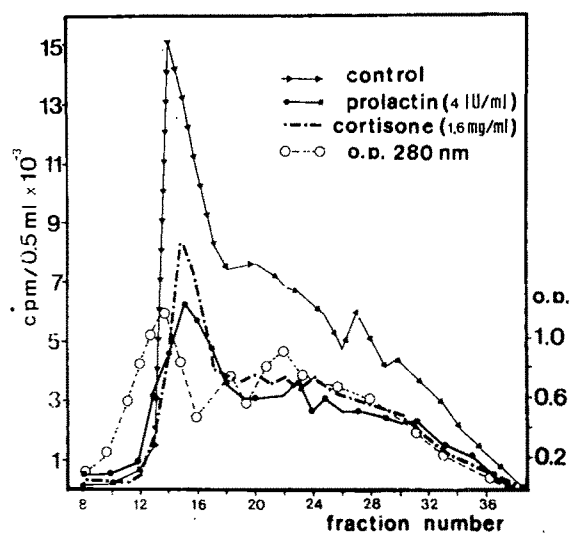


**Fig. 3.** Gel filtration on Sephadex G-200 of  $105,000 \times g$  tissue supernatant of human fetal lung fragments incubated in vitro with prolactin (4 mIU/ml) and cortisone (1.6 mg/ml). The radioactivity and optical density (O.D.) of each fraction are indicated on the ordinates. A total of 4 ml of tissue supernatant with specific radioactivity of 439,710 cpm/mg for the control sample, 184,470 cpm/mg for the prolactin-treated group, and 324,065 cpm/mg of proteins for the cortisone-treated group was applied separately.

A total of 4 ml of the  $105,000 \times g$  supernatant fraction (protein concentration, 4 mg/ml) was examined by gel chromatography from control and prolactin-treated incubations. Fractions of 2 ml were collected at a rate of 15 ml/hr. Aliquots of 0.5 ml from each effluent fraction were used to assess radioactive content.

**Interpretation of data.** All samples were assayed in duplicate, and the coefficient of variation was less than 15%. All values represent the mean of duplicate incubations. The statistical significance of the difference between sample means was determined by Student's test.

## Results

**In vitro incorporation of  $^{14}C$ -leucine into human fetal lung proteins.** The incorporation of  $^{14}C$ -leucine into midgestation human fetal lung tissue fragments was measured after 15, 30, 60, and 180 minutes of incubation. As shown in Fig. 1, the incorporation of radioactivity into TCA-precipitable proteins in the  $12,000 \times g$  supernatant fraction was very rapid and reached a value of 80,000 dpm/mg of proteins after 3 hours of incubation. The release of  $^{14}C$ -proteins into the incubation medium increased in a linear manner during the incubation period and made up 5% to 8% of the total radioactivity in this fraction after 3 hours of incubation. The particulate fraction ( $12,000 \times g$  pellet)

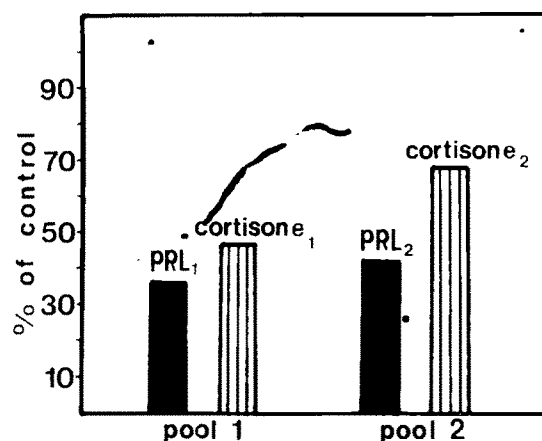


**Fig. 4.** The radioactivity of pool 1 and pool 2 after gel filtration on Sephadex G-200. The results are expressed as a percentage of control. Pool 1 = fractions 12 to 19; pool 2 = fractions 19 to 36. Prolactin = 4 mIU/ml. Cortisone = 1.6 mg/ml.

contained about 50% of the total radioactivity recovered in soluble proteins.

**Effect of hormones on protein synthesis by the human fetal lung.** In order to study the effect of hormones present in the amniotic fluid on protein synthesis in human fetal lung tissue, the incorporation rate of  $^{14}C$ -leucine into lung proteins was assessed after the addition of the following hormones to the incubation medium: prolactin, insulin, cortisone, HPL, PGF<sub>2 $\alpha$</sub> , triiodothyronine, (T<sub>3</sub>), and thyroxine (T<sub>4</sub>).

**Fetal lung tissue at 18 weeks of gestation.** Lung tissue slices were incubated with prolactin and different doses of cortisone for 3 hours. The results are illustrated in Fig. 2. A significant inhibition of  $^{14}C$ -leucine incorporation into the  $105,000 \times g$  tissue supernatant fraction after both prolactin and cortisone treatment was seen. This inhibitory effect of hormone treatment was dose dependent. The minimal effective dose for prolactin was 4 mIU/ml and that for cortisone was 1.6 mg/ml. The addition of HPL, T<sub>3</sub>, T<sub>4</sub>, and PGF<sub>2 $\alpha$</sub>  to the incubation medium did not affect the synthesis of fetal lung proteins.

**Gel filtration on Sephadex G-200 column.** The elution pattern from the  $105,000 \times g$  tissue supernatant fraction of lung fragments from the control and prolactin- and cortisone-treated groups is shown in Fig. 3. Three major peaks of protein were eluted by 0.1M NH<sub>4</sub>HCO<sub>3</sub>. Each of the peaks was pooled into two major fractions and the radioactivity was determined. As indicated in Fig. 4, a significant difference was seen between the control and the prolactin- and cortisone-treated groups. The inhibition of  $^{14}C$ -leucine incorporation into fetal lung protein was more pronounced



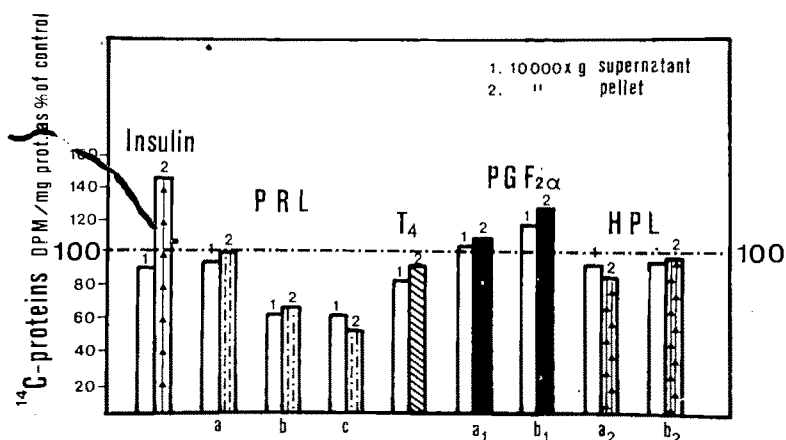


Fig. 5. The incorporation of  $^{14}\text{C}$ -leucine into the  $10,000 \times \text{g}$  tissue supernatant and pellet after in vitro treatment with different hormones. Insulin =  $10 \mu\text{IU/ml}$ . Prolactin = 2, 4, and 8 mIU/ml.  $\text{T}_4$  =  $0.1 \mu\text{g/ml}$ .  $\text{PGF}_{2\alpha}$  = a,  $3 \text{ ng/ml}$ ; b,  $10 \text{ ng/ml}$ . HPL = a,  $1 \mu\text{g/ml}$ ; b,  $5 \mu\text{g/ml}$ .

with prolactin treatment than after cortisone treatment (Fig. 4).

**Fetal lung tissue at 22 to 24 weeks of gestation.** The radioactivity present in the  $10,000 \times \text{g}$  pellet and supernatant fraction after incubation with different hormones in vitro (expressed as a percentage of the control value) is shown in Fig. 5. It is evident that protein synthesis was inhibited in a dose-dependent manner by prolactin treatment and was similar to the effect seen with 16-week-old fetal lung tissue. Maximal inhibition of protein synthesis was about 50% to 60% of the control level, with 4 mIU/ml of prolactin. Insulin treatment increased the radioactivity in the  $10,000 \times \text{g}$  pellet and did not affect the incorporation rate of  $^{14}\text{C}$ -leucine into proteins from the  $10,000 \times \text{g}$  supernatant fraction. High doses of  $\text{PGF}_{2\alpha}$  provoked a slight increase in the radioactivity in the  $10,000 \times \text{g}$  pellet and supernatant fraction. In vitro treatment with HPL,  $\text{T}_3$ ,  $\text{T}_4$ , and lower doses of  $\text{PGF}_{2\alpha}$  had no effect on protein synthesis in the fetal lung in the present study.

In order to confirm the inhibitory effect of the in vitro treatment with prolactin on protein synthesis by the human fetal lung, three independent experiments were carried out (Fig. 6).

The radioactivity incorporated into the protein was measured in the  $10,000 \times \text{g}$  tissue supernatant fraction and incubation medium after in vitro treatment with prolactin ( $4 \text{ mIU/ml}$ ) or HPL ( $4 \mu\text{g/ml}$ ). A statistically significant decrease in protein-bound radioactivity in both tissue and medium was observed after prolactin treatment ( $P = 0.05$ ). HPL treatment, on the other hand, decreased the release of the newly synthesized proteins into the medium but had no effect on protein synthesis.

### Comment

Despite the fact that the slice of human fetal lung tissue used in the present study contained a mixed cell population, it was reasonable to assume that the events measured took place predominantly in cells lining the terminal lung unit, based upon previous light microscopic evidence.

The data obtained show that the human fetal lung from 16 to 24 weeks of gestation rapidly incorporated  $^{14}\text{C}$ -leucine into proteins from the particulate fraction and  $105,000 \times \text{g}$  tissue supernatant fraction in a manner similar to that described by Massaro and associates<sup>13</sup> for the adult rat and rabbit lung tissue. The finding that protein-bound radioactivity in the medium increased during incubation, together with the observation that this time-dependent increase can be slowed down by chilling, indicates that the fetal lung cells are capable of active secretion of proteins. The distribution of radioactivity between protein fractions after gel filtration on Sephadex G-200 revealed three major peaks of radioactivity. The elution profile with Sephadex G-200 chromatography closely resembled the elution profile from a human lung cancer tissue homogenate after gel filtration on Sephadex G-200.<sup>15</sup> Sega and associates<sup>15</sup> have purified and characterized one fetal lung antigen associated with human bronchogenic carcinoma with the use of fetal lung tissue and lung carcinoma tissue. According to our results, this protein, detected at the site of the 7S peak of normal serum, was synthesized in vitro by human lung tissue; a more rapidly labeled 19S protein also was released into the incubation medium.

The addition of prolactin and cortisone to the incubation medium led to a dose-dependent inhibition of total protein synthesis by the human fetal lung in vitro.

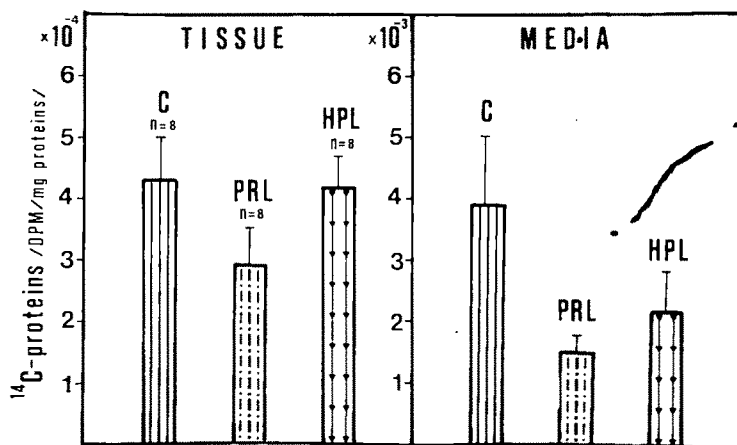


**Fig. 6.** The in vitro incorporation of  $^{14}\text{C}$ -leucine into TCA-precipitable proteins from the  $10,000 \times g$  supernatant of a fetal lung tissue homogenate and incubation medium after 3 hours of incubation. These data are the results of four independent experiments with lung tissue from two male fetuses of 22 to 24 weeks of gestation.

The significant decrease in  $^{14}\text{C}$ -leucine incorporation was noticed in proteins eluted from the Sephadex G-200 column in fractions corresponding to the 19S and 7S peaks of normal human sera.

The inhibitory effect of cortisone on protein synthesis by human fetal lung tissue slices in vitro seen in the present studies is compatible with the suppressed cell proliferation in lungs of cortisone-treated fetuses in utero.<sup>7, 9</sup> However, the evidence that prolactin stimulates surfactant synthesis<sup>12</sup> seems to contradict the prolactin-induced inhibition of lung protein synthesis in vitro described in this paper. This difference may be due to the fact that the human fetal lung tissue used in this study was derived from second-trimester fetuses, which is much earlier than the tissue used in other studies on the human fetal lung. Minimally, the effec-

tive inhibitory dose of prolactin corresponded to the range of maternal serum and amniotic fluid prolactin concentrations for the corresponding gestational age. This was confirmed by determination of prolactin concentrations in serum and amniotic fluid of each patient before cesarean section.

The physiologic implications of the present findings are unknown since virtually no information on the nature or role of fetal lung proteins is available.

We wish to thank Dr. Harold Behrman (Yale University) for critical redaction of the manuscript. We also thank the National Institutes of Health, Bethesda, Maryland, for providing prolactin standards. The assistance of the clinical staff at the Clinic of Gynecology and Obstetrics, Faculty of Medicine, Belgrade, is also appreciated.

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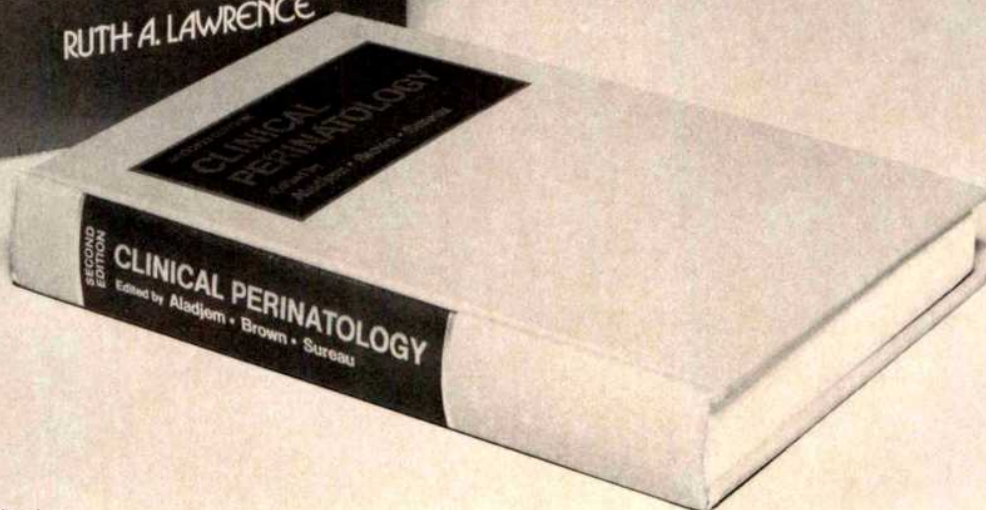
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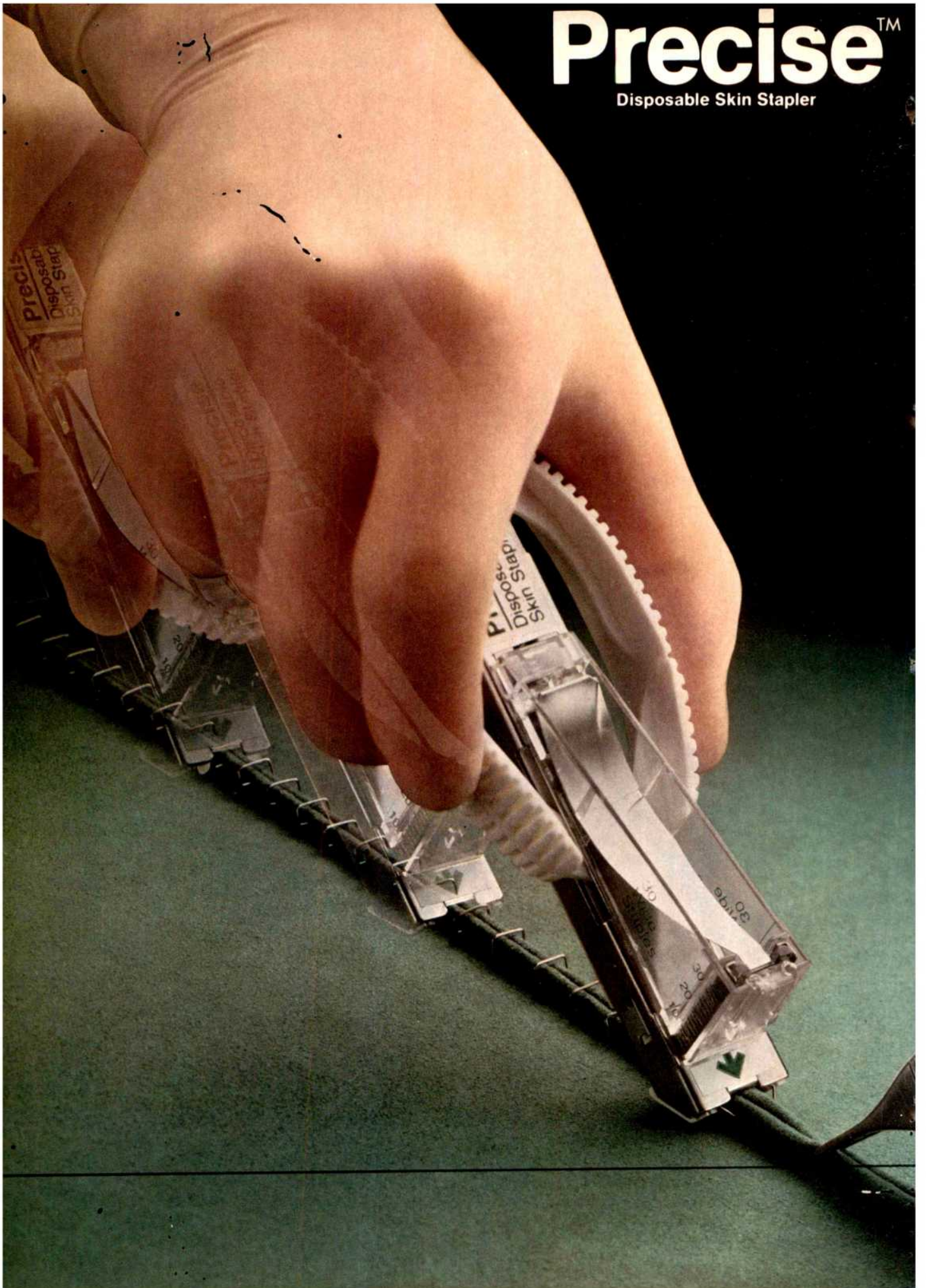
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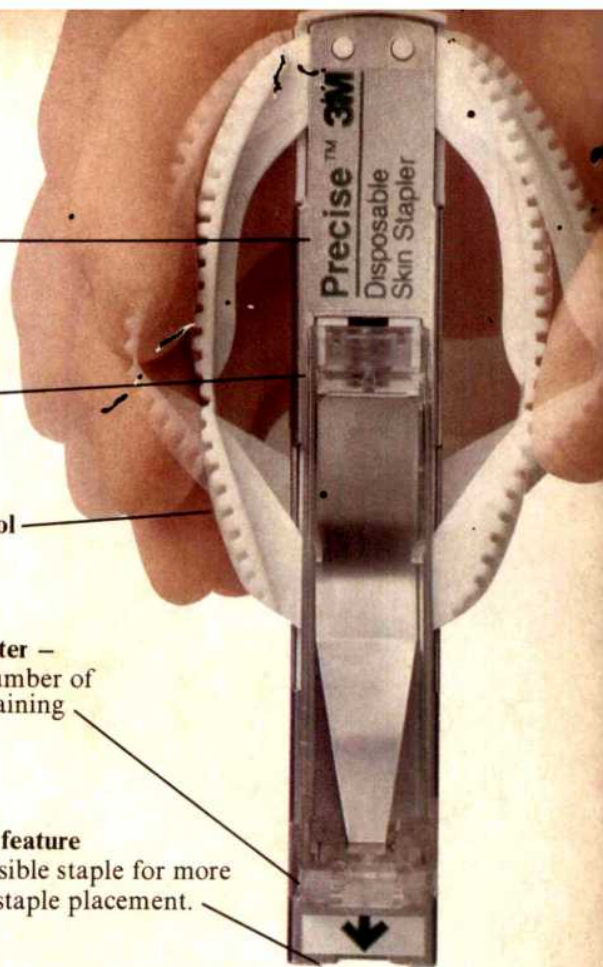
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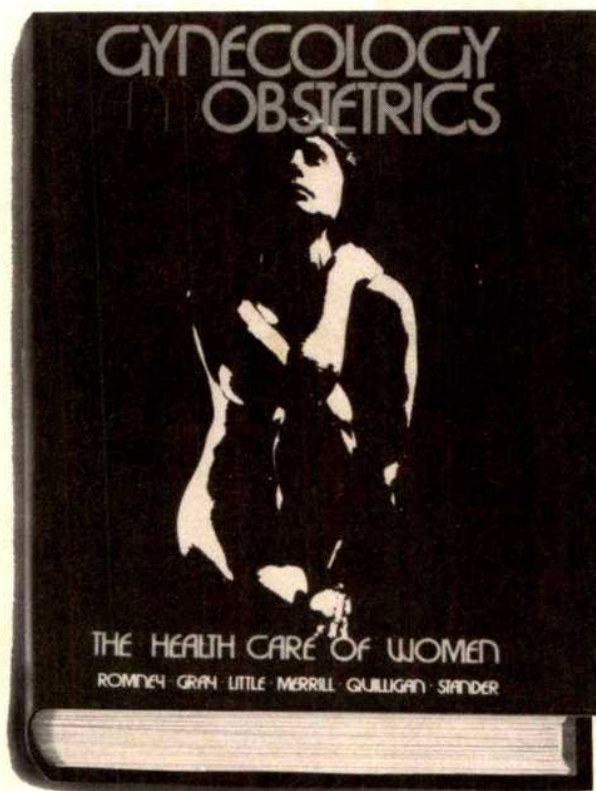
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1) **Thromboembolic Disorders and Other Vascular Problems.** An increased risk of thromboembolic and thrombotic disease associated with use of oral contraceptives is well established. Three principal studies in Great Britain and three in the United States have demonstrated an increased risk of fatal and nonfatal venous thromboembolism and stroke, both hemorrhagic and thrombotic. These studies estimate that users of oral contraceptives are 4 to 11 times more likely than nonusers to develop these diseases without evident cause. In a study of cerebrovascular disorders in women with and without predisposing causes, it was estimated that the risk of hemorrhagic stroke was 2.0 times greater than nonusers and the risk of thrombotic stroke was 4 to 9.5 times greater. An increased risk of myocardial infarction associated with the use of oral contraceptives has been reported. Studies conducted in the United Kingdom found that the greater the number of underlying risk factors for coronary artery disease (cigarette smoking, hypertension, hypercholesterolemia, obesity, diabetes, history of preclampsia/taxemia) the higher the risk of developing myocardial infarction, regardless of whether the patient was an oral contraceptive user or not. Oral contraceptives, however, were found to be a clear additional risk factor. In terms of relative risk, it has been estimated that oral contraceptive users who do not smoke (smoking considered a major predisposing condition to myocardial infarction) are about twice as likely to have a fatal myocardial infarction compared to nonusers who do not smoke. Oral contraceptive users who smoke have about a 5-fold increased risk of fatal infarction compared to users who do not smoke but about a 10- to 12-fold increased risk compared to nonusers who do not smoke. The amount of smoking is a very important factor. British investigators concluded that the risk of thromboembolism including coronary thrombosis is directly related to the dose of estrogen used in oral contraceptives, preparations containing 100 mcg. or more of estrogen were associated with a higher risk of thromboembolism than those containing 50-80 mcg. of estrogen. The relative risk of thromboembolic disease associated with progestin-only oral contraceptives has not been determined. Cases of thromboembolic disease have been reported in women using progestin-only products, and they should not be presumed to be risk-free. The overall excess mortality rate annually from circulatory diseases for oral contraceptive users was estimated to be 20 per 100,000 (ages 15-35, 5/100,000; ages 35-44, 33/100,000; ages 45-49, 140/100,000); the risk being concentrated in older women, in those with a long duration of use, and in cigarette smokers. The highest risk was found in heavy cigarette smokers (15 or more cigarettes per day) who used oral contraceptives and were aged 40 or older. Women who smoke should be advised not to use oral contraceptives. The use of oral contraceptives in women over age 40 with other risk factors is not recommended. The mortality associated with all of the methods of birth control is low compared to the risk of childbirth, with the exception of oral contraceptive users who smoke and are over age 40. The lowest mortality is associated with the condom or diaphragm backed up by early abortion. The risk of thromboembolic and thrombotic disease associated with oral contraceptives increases with age after approximately age 30 and for myocardial infarction is further increased by hypertension, hypercholesterolemia, obesity, diabetes, or history of preclampsia/taxemia, and especially by cigarette smoking. The physician and the patient should be alert to the earliest manifestations of thromboembolic and thrombotic disorders (e.g., thrombophlebitis, pulmonary embolism, cerebrovascular insufficiency, coronary occlusion, retinal thrombosis and mesenteric thrombosis). Should any of these occur or be suspected, the drug should be discontinued immediately. If feasible, oral contraceptives should be discontinued at least 4 weeks before surgery of a type associated with an increased risk of thromboembolism or prolonged immobilization. 2) **Ocular Lesions.** There have been reports of neuro-ocular lesions such as optic neuritis or retinal thrombosis associated with the use of oral contraceptives. Discontinue the medication if there is unexplained sudden or gradual, partial or complete loss of vision; sudden onset of proptosis or diplopia; papilledema, or retinal vascular lesions, and institute appropriate diagnostic and therapeutic measures. 3) **Carcinoma.** Long term administration of either natural or synthetic estrogen in certain animal species increases the frequency of carcinoma of the breast, cervix, vagina and liver. In humans, one study investigated the first 21 cases of endometrial adenocarcinoma in women on oral contraceptives reported to a registry. Of those women without predisposing risk factors for this disease, nearly all occurred in women who had used a sequential oral contraceptive. No evidence has been reported suggesting an increased risk of endometrial cancer in users of conventional combination or progestin-only oral contraceptives. No increase in breast cancer in women taking oral contraceptives has been reported although one study reported an increased risk of breast cancer in subgroups of women treated using oral contraceptives with documented benign breast disease. There is at present no confirmed evidence from human studies of an increased risk of cancer associated with oral contraceptives. Close clinical surveillance of all women taking oral contraceptives is essential. In all cases of undiagnosed persistent or recurrent abnormal vaginal bleeding, appropriate diagnostic measures should be taken to rule out malignancy. Women with a strong family history of breast cancer or who have breast nodules, fibrocystic disease or abnormal mammograms should be monitored with particular care if they elect to use oral contraceptives. 4) **Hepatic Adenoma.** Benign hepatic adenomas have been found to be associated with the use of oral contraceptives. One study reported a higher risk associated with oral contraceptive formulations with high hormonal potency. Although benign and rare, hepatic adenomas may rupture and may cause death through intra-abdominal hemorrhage. This has been reported in short term as well as long term users of oral contraceptives. Two studies relate the risk with duration of contraceptive use, the risk being much greater after 4 or more years of oral contraceptive use. While hepatic adenoma is a rare lesion, it should be considered in women presenting abdominal pain and tenderness, abdominal mass, or shock. A few cases of hepatocellular carcinoma have been reported in women taking oral contraceptives. The relationship of these drugs to this type of malignancy is not known at this time. 5) **Use in Pregnancy, Birth Defects in Offspring and Malignancy in Female Offspring.** Fetal abnormalities have been reported to occur in the offspring of women who have taken progestogens and/or estrogens during pregnancy. It has been shown that females exposed in utero to diethylstilbestrol, a nonsteroidal estrogen, have an increased risk of developing in later life a form of vaginal or cervical cancer that is ordinarily extremely rare. A high percentage of such exposed women (30% to 90%) have been found to have epithelial changes of the vagina and cervix. Although these changes are histologically benign it is not known whether this condition is a precursor of vaginal malignancy. Male children so exposed may develop abnormalities of the urogenital tract. Similar data are not available with the use of other estrogens but it cannot be presumed that they would not induce similar changes. The data suggest that the risk of limb reduction defects in exposed fetuses is somewhat less than 1 in 1000 live births. In the past, female sex hormones have been used during pregnancy in an attempt to treat threatened or habitual abortion. There is considerable evidence that estrogens are ineffective for these indications and there is no evidence from well-controlled studies that progestins are effective for these uses. Increases in chromosomal aberrations have been reported in women who become pregnant soon after ceasing

oral contraceptive therapy. Embryos with these anomalies are virtually always spontaneously aborted. Whether there is an overall increase in spontaneous abortion of pregnancies conceived soon after stopping oral contraceptives is unknown. It is recommended that for any patient who has missed two consecutive periods, pregnancy should be ruled out before continuing the contraceptive regimen. If the patient has not adhered to the prescribed schedule, the possibility of pregnancy should be considered at the time of the first missed period and further use of oral contraceptives should be withheld until pregnancy has been ruled out. If pregnancy is confirmed the patient should be apprised of the potential risks to the fetus and the advisability of pregnancy continuation should be discussed in light of these risks. It is also recommended that women who discontinue oral contraceptives with the intent of becoming pregnant use an alternate form of contraception for a period of time before attempting to conceive. Many clinicians recommend three months. Administration of progestin-only or progestin-estrogen combinations to induce withdrawal bleeding should not be used as a test for pregnancy. 6) **Gall Bladder Disease.** Studies have reported an increased risk of surgically confirmed gallbladder disease appearing after one year of oral contraceptive use and doubling of the risk after 4 or 5 years of use. 7) **Carbohydrate and Lipid Metabolic Effects.** A decrease in glucose tolerance has been observed in a significant percentage of patients on oral contraceptives. For this reason, prediabetic and diabetic patients should be carefully observed while receiving oral contraceptives. Increased serum levels of triglycerides and total phospholipids have been observed in oral contraceptive users. The clinical significance of this observation is unknown at this time. 8) **Elevated Blood Pressure.** An increase in blood pressure has been reported in women receiving oral contraceptives. The prevalence of hypertension in oral contraceptive users may be no higher than nonusers in the first year of oral contraceptive use but increases with longer exposure and in the fifth year of use is two and one-half to three times the reported prevalence in the first year. Women who previously had hypertension during pregnancy may be more likely to develop elevation of blood pressure when given oral contraceptives. 9) **Headaches.** The onset or exacerbation of migraine or development of a new pattern which is recurrent, persistent or severe, requires discontinuation of oral contraceptives and investigation of the cause. 10) **Bleeding Irregularities.** Breakthrough bleeding, spotting and amenorrhea are frequent reasons for patients discontinuing oral contraceptives. In breakthrough bleeding, as in all cases of irregular bleeding from the vagina, nonfunctional causes should be borne in mind. In undiagnosed persistent or recurrent abnormal bleeding from the vagina, adequate diagnostic measures are indicated to rule out pregnancy or malignancy. If pathology has been excluded, time or change to another formulation may solve the problem. Changing to an oral contraceptive with a higher estrogen content, while potentially useful in minimizing menstrual irregularity, should be done only if necessary since this may increase the risk of thromboembolic disease. Women with a past history of oligomenorrhea or secondary amenorrhea or young women without regular cycles may have a tendency to remain anovulatory or to become amenorrheic after discontinuation of oral contraceptives. Women with these preexisting problems should be advised of this possibility and encouraged to use other contraceptive methods. 11) **Ectopic Pregnancy.** Ectopias as well as intrauterine pregnancy may occur in contraceptive failures. However, in oral contraceptive failures, the ratio of ectopic to intrauterine pregnancies is higher than in women not using oral contraceptives since the drugs are more effective in preventing intrauterine rather than ectopic pregnancy. The higher ectopic-intrauterine ratio has been reported with both combination products and progestin-only oral contraceptives. 12) **Breastfeeding.** A small fraction of the hormonal agents in oral contraceptives has been identified in the milk of mothers receiving these drugs. The long-range effect to the nursing infant cannot be determined at this time.

**Precautions:** 1) A complete medical and family history should be taken prior to the initiation of oral contraceptives. Examination should include special reference to blood pressure, breasts, abdomen and pelvic organs, including Papanicolaou smear and relevant laboratory tests. As a general rule, oral contraceptives should not be prescribed for longer than 1 year without another physical examination being performed. 2) Under the influence of estrogen-progestogen preparations, preexisting uterine leiomyomata may increase in size. 3) Patients with a history of psychic depression should be carefully observed and the drug discontinued if depression recurs to a serious degree. 4) Because oral contraceptives may cause some degree of fluid retention, conditions which might be aggravated by fluid retention, such as convulsive disorders, migraine syndrome, cardiac or renal insufficiency or asthma require careful observation. 5) Patients with a past history of jaundice during pregnancy have an increased risk of recurrence of jaundice while receiving oral contraceptive therapy. If jaundice develops in any patient receiving such drugs, the medication should be discontinued. 6) Steroid hormones may be poorly metabolized in patients with impaired liver function and should be administered with caution in such patients. 7) Oral contraceptive users may have disturbances in normal tryptophan metabolism which may result in a relative pyridoxine deficiency. The clinical significance of this is yet to be determined. 8) Serum folate levels may be depressed by oral contraceptive therapy. This may complicate subsequent pregnancy with regard to folate deficiency. 9) The pathologist should be advised of oral contraceptive therapy when relevant specimens are submitted. 10) Certain endocrine and liver function tests and blood components may be affected by estrogen-containing oral contraceptives: a. Increased sulfobromophthalein retention. b. Increased prothrombin and factors VII, VIII, IX, and X. c. Decreased antithrombin III. d. Increased norepinephrine-induced platelet aggregability. e. Increased thyroid binding globulin (TBG) leading to increased circulating total thyroid hormone, as measured by protein-bound iodine (PBI), T<sub>4</sub> by column, or T<sub>4</sub> by radioimmunoassay. Free T<sub>4</sub> resin uptake is decreased, reflecting the elevated TBG. Free T<sub>4</sub> concentration is unaltered. f. Decreased pregnandiol excretion. g. Reduced response to metyrapone test.

**Information for the Patient:** Detailed Patient Labeling has been prepared for use by the patient and has been made available for distribution by the pharmacist.

**Drug Interactions:** Reduced efficacy and increased incidence of breakthrough bleeding have been associated with concomitant use of rifampin. A similar association has been suggested with barbiturates, phenylbutazone, phenytoin sodium and ampicillin.

**Adverse Reactions:** An increased risk of the following serious adverse reactions has been associated with the use of oral contraceptives (see **Warnings**): Thrombophlebitis, pulmonary embolism, coronary thrombosis, cerebral thrombosis, cerebral hemorrhage, hypertension, gall bladder disease, congenital anomalies. There is evidence of an association between the following conditions and the use of oral contraceptives, although additional confirmatory studies are needed: Mesenteric thrombosis, benign hepatomas, neuro-ocular lesions, e.g., retinal thrombosis and optic neuritis. The following adverse reactions have been reported in patients receiving oral contraceptives and are believed to be drug related: Nausea and/or vomiting, usually the most common adverse reactions, occur in approximately 10% or less of patients during the first cycle. (Other reactions, as a general rule, are seen much less frequently or only occasionally.): gastrointestinal symptoms (such as abdominal cramps and bloating), breakthrough bleeding, spotting, change in menstrual flow, dysmenorrhea, amenorrhea during and after treatment, temporary infertility after discontinuance of treatment, edema, chloasma or melasma which may persist, breast changes (tenderness, enlargement, and secretion), change in weight (increase or decrease), change in cervical erosion and cervical secretion, possible diminution in lactation when given immediately postpartum, cholestatic jaundice, migraine, increase in size of uterine leiomyomata, rash (allergic), mental depression, reduced tolerance to carbohydrates, vaginal candidiasis. The following adverse reactions have been reported in users of oral contraceptives, and the association has been neither confirmed nor refuted: Premenstrual-like syndrome, intolerance to contact lenses, change in corneal curvature (steepening), cataracts, changes in libido, chorea, changes in appetite, cystitis-like syndrome, headache, nervousness, dizziness, hirsutism, loss of scalp hair, erythema multiforme, erythema nodosum, hemorrhagic eruption, vaginitis, porphyria, impaired renal function.

**Acute Overdosage:** Serious ill effects have not been reported following acute ingestion of large doses of oral contraceptives by young children. Overdosage may cause nausea, and withdrawal bleeding may occur in females.

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## GYNECOLOGY

### Early menopause and the risk of myocardial infarction

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We evaluated the relation between age at menopause and the risk of nonfatal myocardial infarction (MI) among 121,964 nurses who responded to a mail questionnaire. Of 279 women who reported having been hospitalized for MI, 123 (44%) were postmenopausal (i.e., no longer menstruating) at the time of hospitalization, compared with 1,859 (33%) of 5,580 age-matched control subjects. Among women who became menopausal because of bilateral oophorectomy, the estimated relative risk of MI increased with decreasing age at menopause, and women who underwent bilateral oophorectomy before age 35 were estimated to have a risk of hospitalization for MI approximately 7.2 times (95% confidence interval, 4.5 to 11.4) that of premenopausal women. Hysterectomy without the removal of both ovaries was only weakly associated with an increased risk. The data support the hypothesis that premature cessation of ovarian function increases the risk of non-fatal MI. (AM. J. OBSTET. GYNECOL. 139:47, 1981.)

YOUNG MEN experience higher rates of coronary artery disease than young women. The difference in

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rates decreases with advancing age, suggesting that ovarian function may be protective.<sup>1</sup> In some studies<sup>2-6</sup> women who experienced an early menopause were observed to have higher rates of coronary disease than those with a later menopause, but other studies have shown no such relation.<sup>7-9</sup> The issue remains unresolved. Since the hysterectomy rate among young women has increased in recent years,<sup>10</sup> it is important to clarify the effect of early menopause.

In this report we describe the relation between age at menopause and nonfatal myocardial infarction (MI) among women under 56 years of age. We studied 279 women who had been hospitalized for MI and 5,580 control subjects selected from among 121,964 registered nurses who responded to a mail questionnaire.

#### Subjects and methods

The study population was restricted to married, female, registered nurses, born between 1921 and 1946,



**Table I.** Relation of risk of MI to menopausal status at the time of the hospitalization for MI among 279 case and 5,580 control subjects

| <i>Menopausal status at MI</i>              | <i>Case subjects</i> | <i>Control subjects</i> | <i>RR* estimate</i> | <i>95% Confidence interval</i> |
|---------------------------------------------|----------------------|-------------------------|---------------------|--------------------------------|
| Premenopausal                               | 156                  | 3721                    | 1.0†                | —                              |
| Postmenopausal:                             |                      |                         |                     |                                |
| Bilateral oophorectomy                      | 48                   | 397                     | 2.9                 | 2.1-4.0                        |
| Natural causes                              | 25                   | 693                     | 0.9                 | 0.6-1.3                        |
| Hysterectomy without bilateral oophorectomy | 50                   | 769                     | 1.6                 | 1.1-2.1                        |

\*Relative risk.

†Reference category.

**Table II.** Relation of risk of MI to age at menopause, according to type of menopause

| <i>Type of menopause</i>                            | <i>Age at menopause (yr)</i> |              |              |            |
|-----------------------------------------------------|------------------------------|--------------|--------------|------------|
|                                                     | <i>&lt;35</i>                | <i>35-39</i> | <i>40-44</i> | <i>≥45</i> |
| <i>Bilateral oophorectomy:</i>                      |                              |              |              |            |
| Case subjects                                       | 19                           | 9            | 10           | 10         |
| Control subjects                                    | 63                           | 82           | 116          | 136        |
| RR* estimate                                        | 7.2                          | 2.6          | 2.1          | 1.8        |
| Adjusted† RR estimate                               | 7.7                          | 2.9          | 1.9          | 1.3        |
| <i>Natural causes:</i>                              |                              |              |              |            |
| Case subjects                                       | 1                            | 3            | 7            | 14         |
| Control subjects                                    | 7                            | 49           | 149          | 488        |
| RR* estimate                                        | 3.4                          | 1.5          | 1.1          | 0.7        |
| Adjusted† RR estimate                               | 2.8                          | 1.1          | 0.9          | 0.5        |
| <i>Hysterectomy without bilateral oophorectomy:</i> |                              |              |              |            |
| Case subjects                                       | 10                           | 13           | 18           | 9          |
| Control subjects                                    | 155                          | 250          | 235          | 129        |
| RR* estimate                                        | 1.5                          | 1.2          | 1.8          | 1.7        |
| Adjusted† RR estimate                               | 1.5                          | 1.3          | 1.6          | 1.4        |

\*Relative risk: The 156 premenopausal case subjects and the 3,721 premenopausal control subjects constitute the reference group.

†Adjusted by multiple logistic regression analysis for age at hospitalization for MI, area of residence, year of hospitalization, duration of use of female hormone supplements after menopause, use of oral contraceptives in the month before hospitalization, obesity, cigarette smoking, history of hypertension, history of diabetes, history of elevated cholesterol, history of angina pectoris, and history of parental MI before age 50.

who lived in 11 states (California, Connecticut, Florida, Maryland, Massachusetts, Michigan, New Jersey, New York, Ohio, Pennsylvania, or Texas) and were listed in the 1972 inventory of the American Nurses' Association. In 1976, we mailed introductory letters and questionnaires to all eligible participants. Of the 172,413 nurses who presumably received questionnaires, 121,964 (71%) returned completed forms after three mailings.

The questionnaire included questions on the dates of diagnosis and hospitalization for 11 conditions and illnesses, including MI. Women who were no longer menstruating were asked to provide the age at which menstruation had ceased and the reason for cessation.

**Case and control subjects.** There were 318 subjects who reported having been hospitalized for MI between 1965 and 1977. Of these, there were 173 women who consented to our reviewing their discharge summaries

and for whom a summary was available: MI was the primary discharge diagnosis for 128; other ischemic heart disease, for 22; and diagnoses other than these, for 23.

Of the 318 subjects with MI, 37 reported having been hospitalized in the year in which the menopause had occurred. These women were excluded since we could not determine the time sequence between the menopause and the occurrence of MI. In addition, two postmenopausal women for whom the type of menopause was unknown were excluded, leaving 279 women in the case series.

For each of the 279 cases, 20 control subjects were selected at random from those respondents without a history of MI who were born in the same year as the case subject and whose menopausal status at the date of hospitalization for the MI was known. Thus, the study group consisted of 279 cases and 5,580 age-matched

control subjects; their median age at the time of the MI was 46 years.

**Methods of analysis.** We estimated the ratio of the rate of hospitalization for MI of each group of postmenopausal women compared with the rate of premenopausal women (relative risk)<sup>11</sup> and tested for trend in the estimated relative risks according to age at menopause.<sup>12</sup> Multiple logistic regression analysis, employing the method of maximum likelihood,<sup>13</sup> was used to control simultaneously for the possible distorting effects of age at the time of hospitalization for MI, area of residence, year of hospitalization, duration of use of supplemental female hormones after menopause, oral contraceptive use in the month before the hospitalization, obesity, cigarettes smoked per day, history of hypertension, history of diabetes, history of elevated cholesterol, history of angina pectoris, and history of parental MI occurring before age 50. All variables for both affected women and their age-matched control subjects were defined in relation to the date of hospitalization for MI.

Results

Of the 279 MI cases, 123 (44%) women were postmenopausal (i.e., no longer menstruating) at the time of the MI compared with 1,859 (33%) of 5,580 age-matched control subjects, as shown in Table I. The estimated relative risk for women menopausal because of bilateral oophorectomy compared with that of premenopausal women was 2.9. Hysterectomy with the retention of at least one ovary was associated with a small increase in risk of MI, and there was no association of natural menopause with risk of MI.

Table II gives the estimated relative risk of MI for postmenopausal women in several categories of age at menopause and type of menopause relative to premenopausal women. The relative risk estimates increased with decreasing age at bilateral oophorectomy ( $\chi^2_1 = 12.3$ ,  $p < 0.001$ ), and the estimate for women having bilateral oophorectomy before age 35 compared with premenopausal women was 7.2, with a 95% confidence interval of 4.5 to 11.4. Based on a small number of observations, natural menopause before 35 years of age was associated with an increased risk of MI, but natural menopause at later ages was not. Hysterectomy without bilateral oophorectomy was weakly associated with an increased risk, and there was no evidence of a trend in the estimates according to age at hysterectomy. Similar findings were observed upon simultaneous adjustment, with the use of multiple logistic regression analysis, for all identified potential confounding factors.

The association between early age at bilateral oopho-

**Table III.** Relation of risk of MI to age at menopause due to bilateral oophorectomy, according to length of interval between bilateral oophorectomy and hospitalization for MI

| Interval between bilateral oophorectomy and MI (yr) | Age at menopause due to bilateral oophorectomy (yr) |       |       |     |
|-----------------------------------------------------|-----------------------------------------------------|-------|-------|-----|
|                                                     | <35                                                 | 35-39 | 40-44 | ≥45 |
| <5:                                                 |                                                     |       |       |     |
| Case subjects                                       | 1                                                   | 1     | 4     | 5   |
| Control subjects                                    | 3                                                   | 13    | 44    | 101 |
| RR* estimate                                        | 8.0                                                 | 1.8   | 2.2   | 1.2 |
| Adjusted† RR estimate                               | 7.5                                                 | 1.7   | 2.2   | 1.5 |
| 5-9:                                                |                                                     |       |       |     |
| Case subjects                                       | 0                                                   | 1     | 5     | 5   |
| Control subjects                                    | 9                                                   | 26    | 57    | 33  |
| RR* estimate                                        | 0                                                   | 0.9   | 2.1   | 3.6 |
| Adjusted† RR estimate                               | 0                                                   | 0.9   | 2.2   | 3.5 |
| 10-14:                                              |                                                     |       |       |     |
| Case subjects                                       | 4                                                   | 4     | 1     | 0   |
| Control subjects                                    | 12                                                  | 34    | 15    | 2   |
| RR* estimate                                        | 8.0                                                 | 2.8   | 1.6   | 0   |
| Adjusted† RR estimate                               | 7.7                                                 | 2.9   | 1.5   | 0   |
| 15-19:                                              |                                                     |       |       |     |
| Case subjects                                       | 7                                                   | 3     |       |     |
| Control subjects                                    | 15                                                  | 8     |       |     |
| RR* estimate                                        | 11                                                  | 8.9   |       |     |
| Adjusted† RR estimate                               | 12                                                  | 8.6   |       |     |
| ≥20:                                                |                                                     |       |       |     |
| Case subjects                                       | 7                                                   | 0     |       |     |
| Control subjects                                    | 24                                                  | 1     |       |     |
| RR* estimate                                        | 11                                                  | 0     |       |     |
| Adjusted† RR estimate                               | 6.9                                                 | 0     |       |     |

\*Relative risk. The 156 premenopausal case subjects and the 3,721 premenopausal control subjects constitute the reference group.

†Adjusted for age at hospitalization for MI (<45, 45 to 49, 50 to 55). For each age stratum, the premenopausal case subjects and control subjects of that age constitute the reference group.

rectomy and increased risk of MI was present among women who were cigarette smokers at the time of the MI as well as among women who were not. The small number of cases precluded a more informative analysis of the association according to smoking and other predisposing factors.

We evaluated whether the length of the interval between menopause and the time of hospitalization for MI modified the effect of early menopause on MI risk. For women who had undergone hysterectomy but retained at least one ovary, the association between age at menopause and MI did not vary appreciably or consistently with length of interval. For naturally menopausal women, the data were too sparse to be informative. Table III shows the distribution of the case subjects and the control subjects in whom bilateral oophorectomy was performed according to age at menopause and the length of the interval between bilateral oophorectomy and hospitalization; relative risk estimates for women in



each subgroup compared with premenopausal women are given. There was evidence for an effect of bilateral oophorectomy before age 40 on risk of MI in all intervals except 5 to 9 years; however, for the intervals of less than 5 years and 5 to 9 years, the estimates were based on a small number of cases. After the subjects were divided according to age at hospitalization, the age-standardized relative risk estimates were similar to the crude estimates; this indicated that the crude association between risk of MI and length of interval was not accounted for by differing age distributions of case subjects and control subjects within the interval subcategories.

### Comment

The data from this study support the hypothesis that premature cessation of ovarian function adversely affects the risk of nonfatal MI. Women menopausal because of bilateral oophorectomy appeared to have a greater risk of hospitalization for MI than premenopausal women of similar ages, and the estimated relative risk of MI increased with decreasing age at bilateral oophorectomy. By contrast, hysterectomy without bilateral oophorectomy was weakly associated with an increase in risk of MI, and the effect was not related to the age at hysterectomy. There were few women who had an early natural menopause, and our findings concerning the relationship between natural menopause and MI were inconclusive.

The effect of early bilateral oophorectomy on the risk of MI was not explained by cigarette smoking, predisposing conditions, female hormone supplements, oral contraceptives, place of residence, or calendar year. Since hysterectomy without bilateral oophorectomy was only weakly associated with an increase in risk of MI, we think it unlikely that the gynecologic surgery or factors leading to surgery themselves account for more than a small part of the observed association between bilateral oophorectomy and MI.

We have no evidence to show whether the participation of women who replied to our questionnaire and who had been hospitalized for MI was biased in regard to their age at menopause. However, respondents and nonrespondents were similar in regard to age distribution and working status.

A spurious association could have arisen if women with an early age at menopause had been more likely to survive an infarction than those with a later age at menopause. This possibility cannot be excluded because our study did not include women whose illness was fatal.

Inaccuracies in the reporting of type of menopause, age at menopause, and MI, if random, would have at-

tenuated an association between early menopause and MI. With regard to type of menopause, women who undergo operative menopause appear to report the nature of the operation with acceptable accuracy. Weiss<sup>14</sup> found that 100% of 33 reports of hysterectomy without bilateral oophorectomy agreed with the hospital record, as did 73% of 83 reports of bilateral oophorectomy. Misclassification of the same magnitude, if present in the current study, would have resulted in moderate underestimation of the relative risk for women menopausal because of bilateral oophorectomy.

In several previous studies,<sup>2-4</sup> women with an early menopause were observed to have greater rates of coronary artery disease than those with a later menopause, but these studies were small and the compared groups differed in factors that may have affected risk. Recently, a similar relation was observed in the Framingham Study,<sup>5</sup> and the data from a cross-sectional study,<sup>6</sup> in which a greater proportion of MI patients than of a population sample were menopausal before the age of 50 years, are also consistent with an adverse effect of early menopause. In contrast, in the largest previous study of menopause and coronary disease,<sup>7</sup> coronary artery disease was observed in 8.2% of 267 women whose ovaries were removed at an early age, compared with 8.1% of 385 women who underwent hysterectomy at similar ages but in whom the ovaries were retained. This study was controlled for several important potentially confounding variables, and diagnostic criteria were well defined. Unexpectedly, however, the rates of coronary artery disease of both the oophorectomy and the hysterectomy groups were more than twice those calculated on the basis of rates among women who had not undergone operative menopause. No explanation for the higher rates in the operative groups was found.

Mortality statistics do not provide support for an effect of menopause on the risk of death from coronary artery disease: Furman<sup>8</sup> and Heller and Jacobs<sup>9</sup> observed that there is no acceleration in the increase in female mortality rates from coronary disease during or after the span of years in which menopause normally occurs. If, however, relatively few women lose ovarian function prematurely and the effect of the loss does not occur until a long time thereafter, an effect of early menopause might not be readily discernible in vital statistics.

Data from two studies suggest that a long interval precedes an adverse effect of early menopause. In an autopsy study,<sup>15</sup> women who had undergone bilateral oophorectomy before 40 years of age exhibited more coronary atherosclerosis than age-matched women with ovaries, but not until at least 10 years after the gynecologic surgery. In a follow-up study of women

who had X-irradiation treatment for metropathia hemorrhagica,<sup>16</sup> a small excess of deaths from coronary disease was observed 10 years following treatment among women whose radiation-induced menopause had occurred before 46 years of age. In the current study, the increased risk of MI associated with bilateral oophorectomy occurring before age 40 was clearly evident 10 or more years later. An effect was also observed shortly after the gynecologic surgery, but this was based on a small number of cases. We could not determine whether onset of the menopause after 40 years of age also increased the risk 10 or more years later because the study was limited to subjects under 56 years of age.

The present data indicate that perhaps one fourth of women undergoing hysterectomy before 40 years of age also have both ovaries removed. In the United States, the rate of operative menopause among young

women has increased in recent years<sup>10</sup>. Between 1965 and 1973, the hysterectomy rate almost doubled for women 25 to 34 years old, from an estimated seven per thousand to 12 per thousand, and for women 35 to 44 years of age, the rate increased from 14 per thousand to 20 per thousand. The health consequences of this trend may not become fully apparent for years to come.

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# Oral contraceptive use, sexual activity, and cervical carcinoma

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Recent studies suggest that long-term oral contraceptive (OC) use may be related to the development of subsequent cervical carcinoma (CaCx). However, the possible confounding effects of sexual activity on this relationship have not been adequately investigated. In this study of 69 patients with CaCx and 216 matched control subjects, data on sexual activity, as well as OC use, were obtained. Sexual activity and OC usage were seen to be positively related, as were sexual activity and risk of CaCx. These two factors were seen to interact to make the risk of CaCx highest in those women who have been most sexually active and who have used OCs for 4 to 6 years. However, after adjustment for sexual factors, the increase in risk of CaCx attributable to OC use was no longer of statistical significance. (AM. J. OBSTET. GYNECOL. 139:52, 1981.)

THE CARCINOGENIC POTENTIAL of various therapeutic hormonal substances has been an attractive epidemiologic theme for some time. Several excellent reviews have summarized the current state of knowledge in this field,<sup>1-4</sup> and to date no clear evidence has been presented to implicate oral contraceptive (OC) steroids in oncogenesis<sup>2-4</sup> in the female reproductive organs.

Recently, indications of a possible association between OC use and cervical epithelial atypias have begun to emerge. Although earlier studies had not demonstrated such a relationship, Ory and associates<sup>5</sup> reported an increased risk of carcinoma in situ and epithelial dysplasia in women using OCs for more than 36 months. Stern and associates,<sup>3</sup> in a prospective study of women with known cervical epithelial dysplasia, demonstrated a greater temporal increase in the severity of dysplasia and conversion to carcinoma among OC users compared to users of other contraceptive methods. Peritz and associates,<sup>7</sup> in a prospective study of 18,000 women in the Walnut Creek Contraceptive Drug Study (CDS) cohort, found a slight positive asso-

ciation between the incidence of cervical carcinoma (CaCx) and the total duration of OC use. Meisels and associates,<sup>8</sup> in a prevalence study of 86,000 French-Canadian women, found a significant excess of cervical epithelial dysplasias in OC users compared to control subjects.

The picture is confounded, however, by the findings of Terris and Oalman,<sup>9</sup> Rotkin,<sup>10</sup> and others, who have repeatedly demonstrated a relationship between several parameters of sexual activity and CaCx, a finding which led Rotkin<sup>10</sup> to the hypothesis that CaCx should be regarded as a venereally transmitted disease. Several of the studies cited above had not taken these factors into consideration.<sup>5-7</sup>

Boyce and associates,<sup>11</sup> in a recently updated retrospective study, found no association between OC use and CaCx in a study which controlled for age at first intercourse and age at first pregnancy. The reverse, however, was reported by Meisels and associates<sup>8</sup> who controlled for age at first coitus.

Because the present level of evidence does not permit conclusions regarding the role of OCs in cervical carcinogenesis, the current study was conceived. We have expanded the work of Peritz and associates<sup>7</sup> within the Walnut Creek CDS cohort to include some relevant covariates of sexual activity.

## Subjects and methods

All women, aged 18 to 54 years, who underwent multiphasic testing between 1969 and 1972, at the Kaiser-Permanente Walnut Creek Medical Center, were subjects in the CDS. These women, who are part

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**Table I.** Study group characteristics for 69 women with cervical cancer and matched control subjects

|                                                | Prevalent      |                  | Incident      |                   | All           |                   |
|------------------------------------------------|----------------|------------------|---------------|-------------------|---------------|-------------------|
|                                                | Cases<br>(26*) | Controls<br>(86) | Cases<br>(43) | Controls<br>(130) | Cases<br>(69) | Controls<br>(216) |
| Age at entry (mean No. of years)               | 36.7           | 36.4             | 32.9          | 32.9              | 34.3          | 34.3              |
| Age at first marriage (mean No. of years)      | 18.8           | 19.9             | 18.5          | 20.6              | 18.6          | 20.3              |
| Age at first pregnancy (mean No. of years)     | 19.0           | 20.8             | 19.5          | 21.1              | 19.3          | 21.0              |
| Age at regular intercourse (mean No. of years) | 19.2           | 20.6             | 18.6          | 20.2              | 18.8          | 20.3              |
| Mean No. of Pap smears                         | 10.8           | 11.6             | 12.2          | 11.6              | 11.6          | 11.6              |
| Mean No. of partners                           | 4.2            | 2.5              | 5.1           | 2.6               | 4.8           | 2.6               |
| Regular intercourse before age 17 (%)          | 11.5           | 8.1              | 16.3          | 6.9               | 14.5          | 7.4               |
| ≥ 11 partners (%)                              | 7.7            | 4.7              | 16.3          | 5.4               | 13.0          | 5.1               |
| Remarried, separated, or divorced (%)          | 53.8           | 24.4             | 53.5          | 24.6              | 53.6          | 24.5              |
| OC use (%):                                    |                |                  |               |                   |               |                   |
| Ever                                           | 57.7           | 64.0             | 86.0          | 71.5              | 75.4          | 68.5              |
| ≥ 1 year                                       | 50.0           | 51.2             | 79.1          | 58.5              | 68.1          | 55.6              |
| ≥ 4 years                                      | 42.3           | 39.5             | 53.5          | 40.8              | 49.3          | 40.3              |
| ≥ 6 years                                      | 23.1           | 22.1             | 23.3          | 23.8              | 23.2          | 23.1              |
| Ever regular use (%):                          |                |                  |               |                   |               |                   |
| Diaphragm                                      | 23.1           | 47.7             | 30.2          | 34.6              | 27.5          | 39.8              |
| Foam/jelly                                     | 19.2           | 29.1             | 18.6          | 20.0              | 18.8          | 23.6              |
| Condom                                         | 15.4           | 19.8             | 4.7           | 19.2              | 8.7           | 19.4              |
| Tubal ligation (%)                             | 7.7            | 47.7             | 30.2          | 34.6              | 27.5          | 39.8              |
| Vasectomy (%)                                  | 3.8            | 16.3             | 4.7           | 21.5              | 4.3           | 19.4              |
| Ever had (%):                                  |                |                  |               |                   |               |                   |
| Monilia                                        | 50.0           | 54.7             | 55.8          | 52.3              | 53.6          | 53.2              |
| Trichomonas                                    | 30.8           | 19.8             | 20.9          | 8.5               | 24.6          | 13.0              |
| Unspecified vaginal infection                  | 7.7            | 14.0             | 14.0          | 20.0              | 11.6          | 17.6              |
| Vaginal herpesvirus                            | 0.0            | 9.3              | 2.3           | 1.5               | 1.4           | 4.6               |
| Cold sores                                     | 46.2           | 44.2             | 41.9          | 36.9              | 43.5          | 39.8              |
| Pelvic inflammatory disease                    | 11.5           | 4.7              | 7.0           | 7.7               | 8.7           | 6.5               |
| Gonorrhea                                      | 3.8            | 4.7              | 7.0           | 2.3               | 5.8           | 3.2               |
| Syphilis                                       | 0.0            | 0.0              | 0.0           | 0.0               | 0.0           | 0.0               |
| Therapeutic abortion                           | 15.4           | 3.5              | 4.7           | 6.2               | 8.7           | 5.1               |
| Protestant (%)                                 | 50.0           | 53.5             | 67.4          | 66.9              | 60.9          | 61.6              |
| Catholic (%)                                   | 26.9           | 24.4             | 14.0          | 20.0              | 18.8          | 21.8              |
| Completed high school (%)                      | 84.6           | 94.2             | 79.1          | 92.3              | 81.2          | 93.1              |

\*Total number.

of a long-term prospective study on the noncontraceptive effects of OCs, have been described elsewhere<sup>12</sup> but may be characterized as a homogeneous, white, middle-class, suburban population.

A subset of this cohort was used by Peritz and associates<sup>7</sup> to analyze the relationship between CaCx and OC use.

As data concerning sexual activity were not collected for the entire CDS study population, an in-depth study of a small subgroup was designed to capture this information. The pathology files at the medical center were screened to identify all cases of CaCx diagnosed between January, 1969, and June, 1976. While recognizing the possibility of varying interpretations in the histologic diagnosis of carcinoma in situ and various degrees of epithelial dysplasia, we chose to accept the pathologist's original diagnosis on the assumption that carcinoma in situ and more severe dysplasias are biologically similar entities and, in agreement with the finding of Peritz and associates,<sup>7</sup> that the association between OC usage and CaCx was not significantly

ameliorated by the addition of cases of dysplasia. During the observation period of up to 7½ years, 72 cases of CaCx were identified, of which 70 were in situ and two were invasive lesions. Of these, 27 cases were diagnosed as a result of follow-up of a suspicious Papanicolaou smear obtained at entry into the CDS. These women were considered to be prevalent cases, and this group was analyzed separately. The remaining 45, the incident cases, were women whose entry Papanicolaou smears were negative.

Approximately 10,000 women actively participating in the CDS in January, 1977, who, according to their CDS questionnaire, had never had an abnormal Papanicolaou smear or a hysterectomy, were considered as possible controls for these cases.

From this group, three controls were selected for each case by matching age, study entry date, number of Papanicolaou smears at entry, marital status, religion, education, parity, and year of menopause (if relevant).

All case and control subjects were interviewed in the gynecology clinic or by telephone and answered a brief



**Table II.** Mean values of some indicators of sexual activity

|                                    | Never used OCs |          | OC users |          |
|------------------------------------|----------------|----------|----------|----------|
|                                    | Cases          | Controls | Cases    | Controls |
| Age at first intercourse (years)   | 18.5           | 19.8     | 18.0     | 19.6     |
| Age at regular intercourse (years) | 19.3           | 20.1     | 18.7     | 20.2     |
| No. of partners                    | 3.4            | 2.1      | 4.9      | 2.6      |
| Total infections* (%)              | 13             | 54       | 80       | 53       |

\*Ever had Trichomonas, unspecified vaginal infection, pelvic inflammatory disease, vaginal herpesvirus, or gonorrhea.

questionnaire designed to update contraceptive and other relevant information, as well as to obtain the missing information on sexual activity. In order to ensure that case and control subjects were comparable with respect to observation time, the control subject's history was terminated at the date of diagnosis of the case subject with whom she was matched.

A detailed literature search suggested that two groups of variables were relevant to risk of CaCx, those related to sexual behavior and those which measure socioeconomic status.<sup>13, 14</sup>

Because of the relatively homogeneous study population, it was not possible to assess the effects of socioeconomic factors in detail. However, both religion and educational level were included in the analysis as socioeconomic variables.

According to the literature, many measures of sexual activity are of interest in the study of CaCx. Most authors agreed on the relevance of age at first marriage, age at first intercourse, number of marriages, and total number of sexual partners,<sup>9, 13, 14</sup> as well as the frequency of various venereal infections, including herpesvirus type II,<sup>15</sup> gonorrhea, syphilis, and Trichomonas.<sup>16</sup> In addition, we ascertained age at regular intercourse, which we arbitrarily defined as at least once a week.

**Statistical analysis.** By means of a technique adapted from Miettinen,<sup>17</sup> the following analysis was carried out: (1) Via linear discriminant analysis, a multivariate score, combining all significant risk factors, was obtained that measured how "caselike" each subject was. We combined indicators of sexual activity and called this measure the sexuality index. This index was used in subsequent analyses because of its simplicity and its ability to separate cases and controls nearly as well as the complete discriminant function. (2) The population was stratified into several levels on the basis of the sexuality index. Within each stratum, the risk of CaCx was assumed to be constant. (3) Estimates of relative risk

(incremented odds ratios) were obtained within each stratum for all OC users compared to nonusers, as well as for short-term, intermediate, and long-term OC users separately. (4) Finally, for each OC use group, a pooled estimate of relative risk was obtained, with confidence intervals, by means of both the incremented mean odds ratio and the method of Mantel and Haenszel.\*<sup>18</sup>

## Results

**Prevalent versus incident cases.** The major difference between prevalent and incident cases was the percentage of those ever having used OCs in each group: 86% among the incident cases and only 58% among the prevalent cases (Table I). This disparity was probably in part the result of ascertainment bias that arises because CaCx is more likely to be found among women who are screened often. Women frequently screened were more likely to be OC users because screening by Papanicolaou smear was required in this population in order to renew an OC prescription. Therefore, these women were more likely to appear as incident cases. In contrast, the prevalent cases were detected at entry, when all women except those recently screened received a Papanicolaou smear. Therefore, this group was likely to have an excess of women who neglected to have a Papanicolaou smear; these women were thus less likely than the rest of the population to be OC users. This argument was supported by a chart review. Of the 54 women with an average interval between Papanicolaou smears of over 18 months, only 5% used OCs for 12 or more months during the period of observation, as contrasted with 47% who had never used OCs. We recognize that unequal Papanicolaou intervals may introduce a bias of ascertainment which may result in an overestimate of risk for OC users.

**Sexuality index.** To unravel the effects of the 12 noncontraceptive variables most clearly related to sexual activity, a stepwise discriminant analysis was carried out. This analysis was done to find the minimal linear combination of sex-related variables which best separated case subjects from control subjects. For this purpose two variables proved almost as effective as the use of all 12 variables† (Tables I and II).

The most significant sex-related factor in distinguishing case subjects from control subjects was the total

\*As the estimates obtained by these two methods were in close agreement and as estimates of standard error are available for the former only, we report only the incremented mean odds ratio.

†Sexuality index = 1.91 Partners + 1.08 Age, where Partners is a code ranging from 1 to 5 for number of partners and Age is a code ranging from 1 to 5 for age at regular intercourse.

**Table III.** Relative risk estimates by oral contraceptive use and sexuality index\* (mean odds ratio)

| OC use             | Sexuality index in prevalent cases (26) |        |      |      | Sexuality index in incident cases (43) |        |      |      |
|--------------------|-----------------------------------------|--------|------|------|----------------------------------------|--------|------|------|
|                    | Low                                     | Medium | High | Any  | Low                                    | Medium | High | Any  |
| Low (<4 years)     | 0.59                                    | 0.68   | 0.35 | 0.51 | 0.86                                   | 1.95   | 2.69 | 1.63 |
| Medium (4-6 years) | 0.22                                    | 2.35   | 0.78 | 1.09 | 1.56                                   | 3.75   | 3.37 | 2.75 |
| High (>6 years)    | 0.66                                    | 2.35   | 0.21 | 0.81 | 0.81                                   | 1.59   | 2.33 | 1.46 |
| Any OC use         | 0.37                                    | 1.66   | 0.30 | 0.59 | 0.88                                   | 2.11   | 2.78 | 1.68 |

\*No entries significantly different from 1.0 at  $p < 0.05$  level.

number of sexual partners. The mean number of partners for all case subjects was about twice that for control subjects, with the difference slightly more marked among incident (5.1 versus 2.6) than among prevalent (4.2 versus 2.5) case subjects and their control subjects. When the percentage of cases in the group with 11 or more partners was examined, the case-control difference in the incident group was much more marked (16.3% versus 5.4%) than that in the prevalent group (7.7% versus 4.7%).

The frequency of subjects who had ever been remarried, divorced, or separated was about twice as great among all case subjects (53.6%) as among all control subjects (24.5%). However, this number is directly related to the total number of sexual partners and could not be included separately in the analysis.

The second of the two variables used to summarize sexual activity in the sexuality index was age at regular intercourse.\* The mean age at onset of regular intercourse was about 1½ years earlier for case subjects than for their control subjects and was consistent with the earlier age at which case subjects married. Furthermore, about twice as many case subjects began regular intercourse before 17 years of age than did control subjects (14.5% versus 7.4%). These differences were somewhat more marked among incident than prevalent cases. The other sex-related variables, particularly vaginal infections (either separately or in combination) and number of therapeutic abortions, were not consistent enough to be helpful as discriminating variables, although we found a history of *Trichomonas* nearly twice as frequently in case subjects as in control subjects.

**Other risk factors.** In addition to the variables which measure sexual activity and those describing OC use, many variables were included as potential risk factors. Largely controlled by matching, and therefore not significant in the analysis, were variables including age, number of Papanicolaou smears, number of pregnancies, and years of follow-up.

\*Age at regular intercourse and age at first intercourse were highly correlated ( $r = 0.80$ ), but the former proved to be a slightly more useful risk factor in this population.

The two socioeconomic measures available in this population, religion and education, were not significant risk factors, although case subjects proved slightly less educated than their control subjects.

With regard to variables relating to other contraceptive methods, a deficit of sterilizations among the subjects was seen, as well as a deficit of women who had ever regularly used "barrier methods" of contraception (diaphragm, foam or jelly, condom). That these differences persist even in the prevalent group, which has fewer OC users, suggests that this is more than an artifact.

**OC use.** Because users of OCs were more frequently screened for CaCx than were nonusers, an OC user who was a CaCx case was more likely to be an incident than a prevalent case. Therefore, we could expect a smaller proportion of OC users among the prevalent cases (relative risk  $< 1$ ) and a greater proportion among the incident cases on the basis of this differential screening rate alone. The overall relative risk of CaCx among prevalent cases was only 0.59, whereas for incident cases the summary estimate was 1.68; neither of these was significantly different from 1.00 ( $p \leq 0.05$ ) (Table III).

**OC use and sexual activity.** The primary question this study was designed to answer was how OC use related to sexual activity and whether such a relationship clarified the previously observed increase in relative risk of CaCx associated with OC use.

The sexuality index was plotted as a function of increasing OC use (Fig. 1). Both the incident and prevalent cases and their controls exhibited some increase in the index with additional OC use, but the greatest effect was seen among the case subjects who were OC users. This effect can be seen by examining each sex-related variable separately for OC users and nonusers (Table II). For each variable relating to sexual behavior, the case subjects who were OC users were most extreme. The effects of sexual behavior and OC use thus appear to interact, so that the most sexually active women who are also OC users are at highest risk of developing CaCx.

**Duration of OC use.** We observed in this population



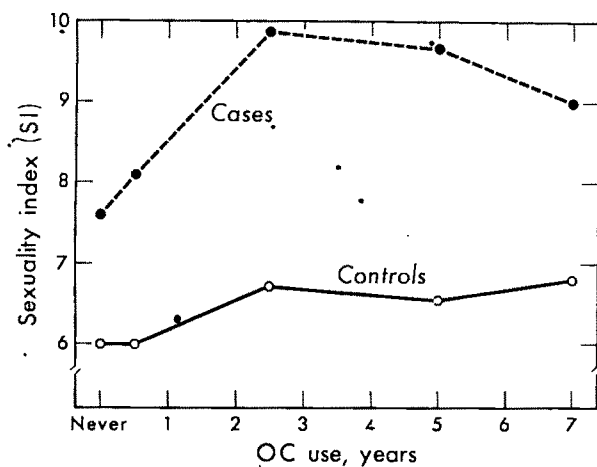


Fig. 1. Sexuality index (SI) as a function of OC use in years.

a trend among incident cases in the effect of OCs on risk of CaCx with duration of OC use, even after we adjusted for sexual indicators (Fig. 2, Table III). The short-term users had only a slight increase in relative risk; the risk then increased slightly, reaching a maximum in those using OCs from 4 to 6 years, and declined in the long-term users (over 6 years). In our population, one third of all OC users were long-term users, so that the decline in relative risk observed among this group was not a random fluctuation caused by small numbers.

The increase in relative risk among incident cases even in the intermediate range of OC use was not, in this population, of great statistical significance ( $0.05 < p < 0.10$ ). However, it is only a slight reduction of the relative risk observed in a nearly identical group of incident cases by Peritz and associates.<sup>7</sup> Even though the individual relative risks are not statistically significant, the pattern of highest risk in the OC users of intermediate duration is itself consistent enough across all levels of the sexuality index to be significant ( $p < 0.05$ ). We conclude that adjusting for sexual factors explains some, but not all, of the relative risk attributable to OC use.

#### Comment

Accumulated evidence from clinical, experimental, and epidemiologic sources suggests a hypothetical model in which there may be initiation of cervical neoplasia in the epithelial transition zone during adolescence or pregnancy, by a venereally transmitted deoxyribonucleic acid factor, viral or other. Promotion of carcinogenesis by the same or other factors may take place during a latent phase of many years.<sup>19</sup> Factors determining total sexual experience, such as age at first

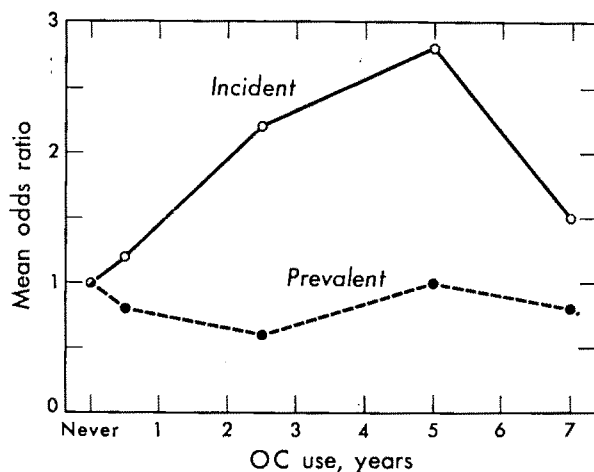


Fig. 2. Risk of cervical cancer (mean odds ratio) as a function of OC use in years.

intercourse, number of partners, and frequency of intercourse may thus be important determinants of the risk of developing CaCx in this model.

A role for therapeutic hormones in the natural history of CaCx has been suggested largely because of the known biologic activity of hormones on target genital tissue and the epidemiologically demonstrated associations between estrogens and other carcinoma of müllerian origin.<sup>1, 2</sup>

Oral contraceptive drugs could, in the hypothetical model, find a role in cancer initiation, promotion, or both. An association between CaCx and increased duration of use of the drug should be demonstrable, provided that adjustment for levels of sexual experience and other relevant covariates has been made. The studies of Ory and colleagues<sup>5</sup> and Peritz and co-workers<sup>7</sup> lacked sufficient sexual data to adjust for these variables. In the studies of Boyce and associates<sup>11</sup> and Meisels and associates<sup>8</sup> only one sexual variable was used.

In this study we have reiterated the association between parameters of sexual activity and cervical neoplasia. In addition, we have shown a direct relationship between increasing OC use and the sexual factors studied.

An association between total duration of OC use and CaCx has persisted among incident cases after adjustment for these sexual factors, but the risk is somewhat less than previously demonstrated and, in this study, is not of statistical significance.

Although assessment of risk at various levels of the sexuality index and OC use is not entirely possible in this study, because of the small size of the study group, the more prominent role of sexuality is suggested by the lower risk of cancer in incident subjects with a low

sexuality index and by the higher risk of cancer in incident subjects with a higher sexuality index at all levels of OC use.

We are aware of the danger of drawing conclusions from a study where a number of biases may be operative. In this study we recognize several. First, we have previously noted a bias in ascertainment of cases; OC users were shown to be more aggressively screened than OC nonusers, with the result that prevalent and incident cases are quite different with respect to both their Papanicolaou smear intervals and their use of oral contraceptives (Table I). Diagnostic bias may be present when the pathologist is consciously aware of the OC status of the patient whose biopsy material is being examined or unconsciously identifies her as an OC user because of cellular changes known to be due to OC effects.

Treatment bias may manifest itself in differential treatment of OC users with biopsies, cervical conization, or cryotherapy, or a greater number of hysterectomies may be performed in OC users with cervical atypias. On the other hand, the opposite situation might prevail if OC users are undertreated because cervical lesions are attributed to OC use and considered to be insignificant. In either event, the number of cases progressing from dysplasia to cancer in situ may be

different for OC users and nonusers. In the former this would lead to an underestimate of the true relative risk, while in the latter an overestimate would result.

The pattern of increasing risk of CaCx with OC use up to 6 years, followed by a reduction in risk, may be the end result of an interaction of several of these biases, resulting in a survivor effect. Long-term observation of cervical atypias by a noninvasive regimen would be necessary to address this question more appropriately.

Although this study leaves the epidemiologic issue of OCs and cervical carcinogenesis unresolved, it may suggest to the clinician that in young women the factors of sexual promiscuity and the prolonged use of OCs may interact over a period of time in a pattern of increasing risk. The benefits of OC use must be judged carefully against these potential risks.

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# Premenopausal hysterectomy and cardiovascular disease

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A premenopausal simple hysterectomy is associated with a threefold increase in the subsequent incidence and prevalence of coronary heart disease during the remaining premenopausal years. During the 10 years following a premenopausal hysterectomy, there is an approximately 4% probability of developing coronary heart disease and a 0.4% probability of dying from it. For every 28 uterine cancer deaths prevented by hysterectomy in 30-year-old women, there is an operative death. To be assured that premenopausal hysterectomies do not cause more deaths from cardiovascular disease than they save by prevention of uterine cancer requires a prospective study of 20,000 women observed until death. Pending further elucidation of this phenomenon, gynecologists should consider advising premenopausal women considering hysterectomy on the risks of coronary heart disease following the procedure. (AM. J. OBSTET. GYNECOL. 139:58, 1981.)

THE BENEFITS of hysterectomy must be weighed against the risks. Apart from the hazard of subsequent bowel obstruction, it has been considered that, with proper psychological screening, the risks of hysterectomy were limited to the operation itself.<sup>1</sup> However, evidence has emerged that premenopausal hysterectomy, even with preservation of one or both ovaries, is associated with a subsequent increased incidence and prevalence of cardiovascular disease.<sup>2-5</sup>

Hysterectomy has beneficial long-term health effects. Following hysterectomy, women will not develop uterine diseases, especially cancers of the cervix and uterine corpus. Any increase in disability and death due to cardiovascular disease must be weighed against decreases in uterine diseases. Evidence relating hysterectomy to cardiovascular disease and uterine cancer is reviewed and compared.

## Methods

Data on uterine cancer and ischemic heart disease mortality are from the Vital Statistics of the United States for the years 1968, 1971, and 1974. Hysterec-

tomy data are from the 1974 Hospital Discharge Survey. Operative mortality rates for hysterectomy are from the Professional Activity Study hospital mortality records for 1974 and 1975.<sup>6</sup>

To make comparable the uterine cancer life-table methodologies,<sup>1,7</sup> a cross-sectional analysis was made in each case with the same data base as in the original life table. The cross-sectional analysis serves as a standard by which the relatively liberal or conservative nature of the life tables can be gauged. Lifetime death rates can be estimated by cross-sectional analysis of deaths occurring in a given year. In 1971, for example, 1.5% of all deaths in women aged 35 and over were attributed to cervical and other uterine cancers. Cross-sectional analysis assumes that, as they pass through successive age groups, women aged 35 in 1971 will take on the proportionate uterine cancer mortality manifested by women of that age group in 1971. Given that assumption, it is estimated that 1.5% of all future deaths of women aged 35 in 1971 will be due to cervical and other uterine cancers.

Menopause occurs with the natural cessation of menses or following bilateral oophorectomy. Following a premenopausal hysterectomy with preservation of ovarian function, a woman remains premenopausal until the ovaries undergo natural menopause. The average age of natural menopause in the United States is 50 years.<sup>8</sup>

The test of statistical significance of data in articles, other than tests performed by the various authors themselves, is the Mantel-Haenszel Chi-square statistic for data stratified by age.

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## Review

**Hysterectomy and uterine cancer mortality.** If a woman without uterine cancer undergoes a hysterectomy, she is no longer at risk of dying from uterine cancer. The magnitude of this benefit is measured by the probability that a woman who does not undergo hysterectomy will eventually die of uterine cancer. Using the 1968 Vital Statistics, Bunker and associates<sup>1</sup> calculated by life-table analysis that 0.2% of women aged 40 in 1968 will eventually die of uterine cancer. By comparison, with a cross-sectional analysis, in 1968, 1.6% of all deaths in women aged 40 or over were due to uterine cancer. Using the 1971 Vital Statistics, Cole and Berlin<sup>7</sup> calculated by life-table analysis that 1.3% of women aged 35 in 1971 will eventually die of uterine cancer. By comparison, with cross-sectional analysis, in 1971 1.5% of all deaths in women aged 35 or over were due to uterine cancer.

Cross-sectional analysis gives the highest estimates of lifetime death rates from uterine cancer; it maximizes the estimated benefits accrued by preventing cancer. Therefore, cross-sectional analysis will be used when the long-term benefits of hysterectomy, mainly the prevention of cancer, are compared with the long-term risks associated with increased cardiovascular disease.

In 1974, 11,455 women aged 30 and over died of cervical and other uterine cancers, which accounted for 1.4% of all deaths in women aged 30 and over. If a woman underwent a hysterectomy at age 30, she averted an estimated 1.4% probability of eventually dying from uterine cancer. Since hysterectomy in the premenopausal woman is associated with an operative mortality of 0.05%,<sup>6</sup> 28 cancer deaths would be prevented for each death caused by the operative procedure itself.

**Hysterectomy and cardiovascular disease.** Death from vascular disease is common whereas death from uterine cancer is, by comparison, uncommon. As suggested by Cole and Berlin,<sup>7</sup> if hysterectomy were to cause, by some unknown mechanism, even a minor increase in death rates from vascular disease, this would more than offset all gains from cancer prevention. In 1974, 36% of all deaths in women aged 30 and over were ascribed to ischemic heart disease. If, for a woman undergoing premenopausal hysterectomy, the probability of dying from ischemic heart disease were to increase to 38%, this 2% increase in mortality would more than offset the 1.4% decrease due to prevention of uterine cancer.

Robinson and associates<sup>2</sup> examined the prevalence of coronary heart disease in women who had undergone prior hysterectomy with preservation of ovarian tissue. All operations were performed before age 45. Among

the 112 women examined, there were five cases of definite coronary heart disease, i.e., myocardial infarction or classical angina pectoris. This was five times more than expected from the age-specific prevalence rates of coronary heart disease observed among women who had not undergone hysterectomy in the Framingham Study ( $P < 0.05$ ).

Ritterband and associates<sup>3</sup> performed a study similar in design to that of Robinson and associates,<sup>2</sup> except that women who had had unilateral oophorectomy were excluded and the diagnosis of coronary heart disease was made blind. Of 369 women who had had simple hysterectomy prior to age 45 and who were evaluated according to Framingham Study criteria, 25 had coronary heart disease. This was 3.3 times more than expected from the age-specific prevalence rates of coronary heart disease among women in the Framingham Study ( $\chi^2 = 23$ ,  $P < 0.0005$ ).

Weiss<sup>4</sup> compared women with aortoiliac occlusion with a control group of women also seen by a vascular surgeon. Among the 54 cases, nine patients had undergone hysterectomy before age 43, with preservation of one or both ovaries. This was 2.6 times more than expected as compared to the controls ( $\chi^2 = 7.6$ ,  $P < 0.01$ ). The relative odds that women who had had premenopausal hysterectomy would develop subsequent aortoiliac occlusion was approximately three to one, as compared to the control group.

Finally, the Framingham Study included its own comparisons.<sup>5</sup> A population-based cohort of 2,873 women was analyzed prospectively with 97% determination of eventual menopausal status, whether natural or surgical; the extent of surgery was determined from hospital operative records in 84% of cases. For ages 45 to 54, women with premenopausal hysterectomy had relative odds of new-onset coronary heart disease 2.7 times greater than those experienced by premenopausal women the same age ( $P < 0.01$ ). The relative odds ratio was the same, whether or not the ovaries were preserved. No myocardial infarctions or coronary heart disease deaths were observed among premenopausal women aged 40 to 54 during 8,384 person years of observation; 10 such events were observed among women aged 40 to 54 during 6,848 person years of observation following premenopausal hysterectomy, with and without preservation of ovaries.

In the Framingham Study the incidence of new coronary heart disease in women following premenopausal hysterectomy, with preservation of one or both ovaries, was four new cases per thousand women per year.<sup>5</sup> In 10% of new cases, the patients died. Thus, it can be predicted that in the 10 years following hysterectomy in premenopausal women approximately 4%



will develop new coronary heart disease; approximately 0.4% will die of coronary heart disease during the first 10 years. Ritterband and associates<sup>3</sup> observed that among women aged 50 or under who had had a simple hysterectomy 10 or more years previously 6% had coronary heart disease; among women undergoing simple hysterectomy prior to age 45, within 10 years following operation 0.5% had died of coronary heart disease.

In 1974, by contrast, death from cervical and other uterine cancers in patients between the ages of 40 and 50 accounted for 0.2% of all deaths in women aged 40 and over. Thus, for women undergoing premenopausal hysterectomy the increased number of deaths caused by coronary heart disease during the premenopausal period is greater than the number of uterine cancer deaths prevented.

It is not clear whether this increased incidence of cardiovascular disease extends into the postmenopausal period.<sup>5</sup> As noted previously, an increase in the lifetime ischemic heart disease death rate from the 1974 level of 36% of all deaths to 38% would more than offset all prevented uterine cancer deaths. To detect with assurance such a small increase requires a large study. Thus, to determine with 90% power, allowing  $P = 0.05$  (one-tailed), that hysterectomy is not associated with an increase in ischemic heart disease deaths from 36% to 38% would require control and case groups of approximately 10,000 subjects each.<sup>9</sup> This assumes the two groups are well matched for other variables and are all observed until death. Furthermore, all 20,000 deaths would have to be verified as to cause by autopsy, since death certificates attributing the cause of death to ischemic heart disease are known to be relatively inaccurate.<sup>10</sup> Until such a study demonstrates the contrary, it must be assumed that an increase in the incidence and prevalence of coronary heart disease in premenopausal women following hysterectomy will eventually lead to an increase in death rates from coronary heart disease, not only in the premenopausal years but in later years as well.

### Comment

Is the association between hysterectomy and cardiovascular disease real? It should be noted that the studies of Robinson and associates,<sup>2</sup> Ritterband and associates,<sup>3</sup> and Weiss<sup>4</sup> were not designed to explore the relationship between hysterectomy and atherosclerosis. Instead, these were studies of the relationship between premenopausal bilateral oophorectomy and atherosclerosis, with patients undergoing noncastrating hysterectomy serving as a control group. Indeed, two of the study designs<sup>2, 3</sup> explicitly assumed that hysterectomy

with preservation of the ovaries would have no effect on atherosclerosis. Therefore, issues arise of possible selection bias<sup>2-4</sup> and appropriateness of control groups,<sup>2, 3</sup> not because of deficiencies in the initial study designs but because these observations were incidental to studies designed for other purposes. These studies serve primarily to confirm and lend weight to the observations of the Framingham Study. In the Framingham Study,<sup>5</sup> an association was observed which has not been explained away as artifact.

Why hysterectomy is associated with a subsequent increased risk of cardiovascular disease is unknown. Two broad possibilities exist.

First, women with an increased risk of cardiovascular disease may have an increased tendency to undergo hysterectomy. If this were so, it would be expected that some of these women at increased risk would not undergo hysterectomy but experience a premenopausal myocardial infarction or coronary heart disease death anyway; likewise, some of those who did undergo hysterectomy could be expected to experience a myocardial infarction prior to the operation. In the Framingham Study, no myocardial infarctions or coronary heart disease deaths were observed in premenopausal women prior to or apart from hysterectomy.<sup>5</sup> The increased rates of cardiovascular disease among women with premenopausal hysterectomy have not been attributable to estrogen use<sup>2-5</sup> or cigarette smoking.<sup>4, 5</sup> Premenopausal women examined prior to hysterectomy had normal serum cholesterol levels, blood pressures, and relative weights when compared to control subjects.<sup>11</sup>

Alternatively, premenopausal hysterectomy may cause, by an unknown mechanism, a subsequent increased risk of cardiovascular disease. The operation itself may cause physiologic or psychological stress which increases risk. The uterus may have an unrecognized hormonal function, e.g., prostaglandin secretion, which, operating independently of or in conjunction with the ovaries, prevents atherosclerosis in premenopausal women. Serum cholesterol levels and weight do not change significantly following premenopausal hysterectomy with preservation of one or both ovaries.<sup>11</sup> Blood hemoglobin levels are positively correlated with hypertension, which, in turn, is a risk factor for cardiovascular disease.<sup>12</sup> However, although premenopausal hysterectomy causes a significant rise in hemoglobin levels, due to cessation of menstrual blood loss, the change is small, a mean rise of 0.62 gm/dl; neither diastolic nor systolic blood pressure changes significantly following premenopausal hysterectomy.<sup>11</sup>

Whatever the relationship between premenopausal hysterectomy and cardiovascular disease, the phe-

nomenon is a new lead in atherosclerosis research which requires further studies to bring about a resolution and understanding.

For the gynecologist, the issue is how to counsel the patient. If hysterectomy was uniformly nonelective, the threat of future coronary heart disease would be unlikely to influence practice. However, some hysterectomies are elective. According to a position paper of the American College of Obstetricians and Gynecologists, only hysterectomies performed for the purpose of sterilization can be considered unequivocally elective (it must not be construed that the College approves of such hysterectomies).<sup>13</sup> In 1970, in a national survey of married women under age 45 with prior hysterectomy, 16% stated that the hysterectomy had been performed for the purpose of sterilization.<sup>14</sup> It is unlikely that this proportion had declined by 1974 since, between 1965 and 1973, the annual age-specific incidence of hysterectomy in women under age 45 increased by over 50%.<sup>15</sup> Therefore, of the 413,000 hysterectomies performed in 1974 on women under age 45, at least 16%, or 66,000, would

be considered elective by the standards of the American College of Obstetricians and Gynecologists. Of these, it can be predicted that by 1984 approximately 4%, or 3,000, will have developed coronary heart disease and 300 will have died of it.

It would be difficult to mount the 20,000-subject study necessary to definitively disprove Cole and Berlin's<sup>7</sup> suggestion that hysterectomy may cause more deaths from heart disease than are prevented by prophylaxis of uterine cancer. Pending further elucidation of the relationship between hysterectomy and cardiovascular disease, gynecologists should consider advising premenopausal women who are considering hysterectomy on the risks of coronary heart disease following the procedure.

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# Ovarian failure in long-term survivors of childhood malignancy

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The frequency and causes of ovarian failure among 182 long-term survivors of childhood cancer were examined. Twenty-two patients (12%) had ovarian failure. Ovarian failure was found in 17 of 25 patients (68%) who had both ovaries within abdominal radiotherapy fields, in five of 35 patients (14%) whose ovaries were at the edge of the treatment field, and in none of 122 patients with one or both ovaries outside of an abdominal treatment field ( $p < 10^{-4}$ ). The odds for ovarian failure in patients with both ovaries in the field are 19.7 higher than those for other irradiated patients (95% confidence interval, 5.3 to 72.8). Covariate and multivariate analyses of tumor type, age at diagnosis, duration of follow-up, abdominal tumor surgery, abdominal radiotherapy, number of chemotherapeutic agents administered, and cumulative doses of several drugs revealed that the location of the ovaries relative to radiation treatment fields was the only risk factor for subsequent ovarian failure. (AM. J. OBSTET. GYNECOL. 139:62, 1981.)

RADIOTHERAPY, chemotherapy, and surgery have contributed to improved prognosis for children with diverse cancers.<sup>1</sup> However, these treatments can also affect normal tissues and produce acute or latent injuries.<sup>2</sup> The late sequelae are now recognized more frequently because of increased numbers of long-term survivors.<sup>2-3</sup> Effects on the reproductive and steroidogenic potential of the gonad after cancer therapy

in children have been reported but neither the incidence nor causes of the impairments have been well defined.<sup>4-5</sup> The present study examines the development of ovarian failure during follow-up in a cohort of 182 women who are long-term survivors of childhood cancer.

## Material and methods

**Study patients.** The tumor registry of the Sidney Farber Cancer Institute in Boston, Massachusetts, provided a list of 208 female patients (1) who had a cancer diagnosed at between 0 and 17 years of age, (2) who were alive and at least 14 years of age at the conclusion of study, (3) who had survived at least 5 years from time of diagnosis, and (4) who had not undergone bilateral ovariectomy.

Demographic and cancer therapy data were obtained on each patient through medical record reviews, clinic interviews, and phone and letter contacts. Twenty-six patients were eliminated from further analysis because of inadequate follow-up information such as data on

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current menstrual function. Other than their loss to follow-up, these 26 patients were comparable to the remaining group of 182 study patients (88%).

The mean age at diagnosis of cancer in the 182 patients was 6.9 years (range 0.1 to 17 years) and the mean number of years of follow-up since diagnosis was 16.4 years (6 to 33 years). Tumor diagnoses were distributed as follows: Wilms' tumor (53 patients), lymphoma (41), soft-tissue sarcoma (24), neuroblastoma (19), osteogenic sarcoma (17), leukemia (10), dysgerminoma (4), renal cell carcinoma (3), thyroid carcinoma (3), ependymoma (2), and one each of six other tumors.

#### **Therapies.**

**Radiation therapy.** Ninety-four of the 182 patients (52%) received fractionated external beam radiotherapy to the abdomen. Available individual radiotherapy field diagrams were utilized to classify the location of each ovary as within, at the edge of, or outside of the irradiated fields with the aid of standardized age-correlated body mannequins; more precise data on actual ovarian tissue doses could not be calculated. All patients who had received whole pelvic and whole abdominal field irradiation were said to have both ovaries within the field. For patients treated prior to 1968 with a 200 kV(p) orthovoltage unit, an ovary located within 10 cm of the margin of a large ( $\geq 10$  by 10 cm) treatment field or located within 3 cm of the margin of a smaller ( $< 10$  by 10 cm) field was considered to be at the edge of the field. An ovary located beyond these ranges was considered to be outside the field. After 1968, when 4 or 8 MeV linear accelerators were used, an ovary within 1 cm of the field margin was considered at the edge and beyond 1 cm was outside the field. When the relative location of the two ovaries differed, the location which provided minimum exposure was used for analysis of association with ovarian failure.

An estimated ovarian tissue dose (OTD) was calculated for each ovary based on 100% of the recorded abdominal midplane dose for ovaries within the field, approximately 10% for ovaries at the edge, and approximately 1% for those outside. Contributions from multiple fields or multiple therapy courses were added. The estimated mean OTDs were approximately 3,200 rads (range 1,200 to 5,000) for the 25 patients with both ovaries inside the field, 290 rads (range 90 to 1,000) for the 35 patients with at least one ovary at the edge of the field, and 54 rads (5 to 150) for the 34 patients with at least one ovary outside the abdominal radiation field.

The charts for 59 patients who received radiotherapy to nonabdominal sites were also reviewed. The es-

timated OTD from scatter radiation for these patients was minimal ( $< 20$  rads). These 59 patients plus the 29 who received no radiotherapy were considered to have received no abdominal irradiation.

**Chemotherapy.** Twenty-one of the 182 patients (12%) received no chemotherapy, and 86 (47%) received one agent. Seventy-five patients received multiple agents, either sequentially or in combination: two agents were given to 42 patients and three or more agents were used in 33 patients. Eighteen different agents were employed, and the cumulative milligram dose of the six drugs utilized most frequently was calculated. For each drug, comparison was made of dose administered to those patients with ovarian failure and those without. Analysis of the effects of multiple-drug regimens was primarily based upon the number of agents administered because of a wide diversity of drug combinations, doses, and treatment schedules.

**Surgery.** One hundred six patients underwent abdominal surgery. Nine of these also received abdominal radiotherapy, nine received chemotherapy, and 83 received both forms of therapy. In 17 patients surgery involved the reproductive tract: unilateral ovariectomy (five patients), ovariopexy (nine), and hysterectomy (three).

**Assessment of ovarian failure.** Ovarian failure is defined as amenorrhea accompanied by persistent elevation of both serum gonadotropins: follicle-stimulating hormone (FSH)  $> 40$  mIU/ml, luteinizing hormone (LH)  $> 25$  mIU/ml, and FSH/LH ratio  $> 1$ .<sup>6</sup> FSH and LH assays were performed by double-antibody radioimmunoassay on plasma obtained prior to institution of hormonal therapy or at least three weeks after its discontinuation.<sup>7</sup> In two clinically hypogonadal amenorrheic patients, diagnosis of ovarian failure was made through a finding of persistently elevated urinary gonadotropin titers of  $> 200$  muu/24 hours  $\times 3$ .<sup>8</sup>

Primary amenorrhea is defined as the lack of spontaneous onset of menstrual function (1) by 14 years of age in the absence of development of secondary sexual characteristics or (2) by 16 years of age with secondary sexual development.<sup>6</sup> Secondary amenorrhea is cessation of spontaneous menstrual function for greater than 6 months.<sup>6</sup> In all study patients with secondary amenorrhea, the abnormality has lasted more than 5 years and has persisted either until the present or until the initiation of replacement hormonal therapy.

**Statistical analysis.** The data were analyzed to determine the differences between the groups of patients with and without ovarian failure. Chi-square analyses were used for categorical data such as radiation exposure and log-rank statistics were used to compare the



**Table I.** Abdominal radiotherapy and chemotherapy for childhood cancer and subsequent ovarian failure in 182 successfully treated patients

| Drug therapy         | Fraction (%) of patients with ovarian failure by ovarian location relative to the radiation field* |              |               |              |
|----------------------|----------------------------------------------------------------------------------------------------|--------------|---------------|--------------|
|                      | No abdominal radiotherapy                                                                          | Out of field | Edge of field | Within field |
| No chemotherapy      | 0/13 (0%)                                                                                          | 0/4 (0%)     | 0/2 (0%)      | 1/2 (50%)    |
| Chemotherapy:        | 0/75 (0%)                                                                                          | 0/30 (0%)    | 5/33 (15%)    | 16/23 (70%)† |
| Single agents        | 0/35 (0%)                                                                                          | 0/22 (0%)    | 4/20 (20%)    | 4/9 (44%)†   |
| Two agents           | 0/26 (0%)                                                                                          | 0/6 (0%)     | 0/8 (0%)      | 2/2 (100%)   |
| Three or more agents | 0/14 (0%)                                                                                          | 0/2 (0%)     | 1/5 (20%)     | 10/12 (85%)  |
| Total‡               | 0/88 (0%)                                                                                          | 0/34 (0%)    | 5/35 (14%)    | 17/25 (68%)† |

\*The ovary with minimum radiation exposure was outside of, at the edge of, or within the radiation field and received estimated mean doses of 3,200, 290, and 54 rads, respectively.  
†Includes two patients receiving actinomycin D and one patient receiving nitrogen mustard for less than 1 week each.  
‡Chi-square value = 90; 3 degrees of freedom ( $p < 10^{-4}$ ).

distributions for continuous variates such as age and drug doses.<sup>9</sup> A logistic regression model was used to simultaneously evaluate several factors which could affect the incidence of ovarian failure among irradiated patients.<sup>10</sup> Factors which were considered for this analysis were ovarian radiation exposure (categorized as in, at the edge of, or outside of the field), number of chemotherapeutic agents administered, age at diagnosis, and years of follow-up since diagnosis.

**Results**

Twenty-two of 182 patients (12%) had ovarian failure. The mean LH level was  $75.5 \pm 15$  (SEM) mIU/ml while the FSH level was  $103.8 \pm 8.7$  (SEM) mIU/ml. Eighteen had primary amenorrhea and four had secondary amenorrhea that developed during cancer therapy. All 22 patients with ovarian failure were among the 94 patients who had received abdominal radiotherapy (Table I). Ovarian failure did not develop in 34 abdominal irradiation patients who had at least one ovary outside of the treatment field but did occur in five of 35 patients (14%) with an ovary at the edge of the treatment field and in 17 of 25 patients (68%) with both ovaries within the treatment field ( $p < 10^{-4}$ ). This trend was independent of chemotherapy. No ovarian failure was noted among 88 patients who had not received abdominal irradiation.

Patients with ovarian failure did not differ from the remainder of the patients with regard to mean age at diagnosis ( $7.0 \pm 1.0$  years [SEM] versus  $6.9 \pm 0.4$ ) and years of follow-up since diagnosis ( $13.9 \pm 1.5$  years [SEM] versus  $16.8 \pm 0.5$ ). Analysis of the average cumulative milligram dose of the six most frequently employed chemotherapeutic agents revealed no consistent or significant differences between the groups (Table II).

In addition, neither tumor type nor surgery within the abdomen was independently associated with development of ovarian failure.

Multivariate analysis with the use of logistic regression models was performed to assess the influence of various factors on the occurrence of ovarian failure. The only significant association was with abdominal radiotherapy. Among the irradiated patients, those who had ovaries within the field of radiation had a significant odds ratio of ovarian failure of 19.7 (95% confidence interval 5.2 to 72.8).

**Comment**

The incidence of ovarian failure was 12% among 182 long-term survivors of childhood malignancies. A strong association was found between ovarian failure and intensity of prior ovarian radiation, with 23% of patients demonstrating ovarian failure if they had received any abdominal radiotherapy and 68% experiencing ovarian failure if the ovaries were determined to be within the radiation field. This association remained after covariate and multivariate analyses of several potentially confounding factors, including age at diagnosis, years since diagnosis, abdominal surgery, cumulative drug dose, and number of chemotherapeutic agents.

Abdominal irradiation has been associated with ovarian failure.<sup>11</sup> Indeed, ionizing radiation has been utilized as a therapeutic means of terminating ovarian function.<sup>11</sup> Very few data, however, are available on ovarian function after irradiation in children.<sup>5</sup>

In the present study, the ovaries were categorized as being within the field of radiotherapy, at the edge of the field, or outside treatment fields. The three classes indicate ovarian irradiation generally in doses of thousands, hundreds, and tens of rads, respectively. A high

**Table II.** Median cumulative chemotherapy doses administered to 182 female survivors of childhood malignancy, by radiation exposure and ovarian failure

|                          | Ovarian exposure to abdominal radiotherapy    |                     |                        |                     |                 |                     |
|--------------------------|-----------------------------------------------|---------------------|------------------------|---------------------|-----------------|---------------------|
|                          | None* or out of field<br>(no ovarian failure) |                     | In or at edge of field |                     |                 |                     |
|                          |                                               |                     | No ovarian failure     |                     | Ovarian failure |                     |
|                          | No. of patients                               | Drug dosage<br>(mg) | No. of patients        | Drug dosage<br>(mg) | No. of patients | Drug dosage<br>(mg) |
| <i>Actinomycin D:</i>    |                                               |                     |                        |                     |                 |                     |
| Total†                   | 47                                            | 4.1                 | 22                     | 3.6                 | 9               | 7.2                 |
| Single agent             | 29                                            | 4.3                 | 15                     | 4.4                 | 7               | 6.8                 |
| <i>Chlorambucil:</i>     |                                               |                     |                        |                     |                 |                     |
| Total                    | 26                                            | 1,665               | 4                      | 1,600               | 4               | 996                 |
| Single agent             | 12                                            | 2,810               | 0                      | —                   | 0               | —                   |
| <i>Cyclophosphamide:</i> |                                               |                     |                        |                     |                 |                     |
| Total                    | 13                                            | 20,175              | 3                      | 3,683               | 7               | 8,560               |
| Single agent             | 3                                             | 29,025              | 2                      | 10,324              | 0               | —                   |
| <i>Methotrexate:</i>     |                                               |                     |                        |                     |                 |                     |
| Total                    | 23                                            | 3,705               | 2                      | 2,108               | 6               | 2,194               |
| Single agent             | 6                                             | 4,717               | 1                      | 20                  | 0               | —                   |
| <i>Vincristine:</i>      |                                               |                     |                        |                     |                 |                     |
| Total                    | 16                                            | 45                  | 9                      | 18                  | 10              | 28                  |
| Single agent             | 0                                             | —                   | 0                      | —                   | 0               | —                   |
| <i>Nitrogen mustard:</i> |                                               |                     |                        |                     |                 |                     |
| Total                    | 7                                             | 20                  | 8                      | 31                  | 5               | 30                  |
| Single agent             | 0                                             | —                   | 1                      | 4                   | 1               | 4                   |

\*No abdominal radiotherapy.

†Total number of patients receiving the drug as either a single agent or in combination with other drugs.

range of radiation exposure dose was found to be strongly correlated with ovarian failure. The finding suggests that less radiation exposure to the gonads would decrease the incidence of subsequent ovarian failure.<sup>12</sup> This can often be accomplished successfully by ovariopexy and lead shielding of the ovaries.<sup>12, 13</sup> The techniques of ovarian displacement and shielding, while having no effect themselves on ovarian function,<sup>13</sup> can decrease gonadal irradiation by 80% to 90% in individuals not undergoing whole pelvic or whole abdominal irradiation.<sup>12</sup> Frequent preservation of ovarian function and fertility have been described after ovariopexy when compared to patients undergoing similar cancer therapy without this ovarian reposition.<sup>12</sup>

Gonadal dysfunction has been documented in adult women<sup>4</sup> and in male subjects of all ages<sup>14</sup> after treatment with some chemotherapeutic agents, particularly alkylating agents. This study and other reports<sup>15, 16</sup> indicate that chemotherapy in female children up to 17 years of age is not a major cause of ovarian failure. During the prepubertal period, the relative quiescence of ovarian stromal cells and oocytes may provide some protection against cytotoxic drugs with cell-cycle specific activity. In addition, the larger number of oocytes in children may permit survival of sufficient oocytes to preserve adequate ovarian function during

early adulthood. Further study is needed to ascertain the frequency and risk factors for premature menopause, oligomenorrhea, nonlethal genetic damage to oocytes, and infertility associated with cancer treatment in childhood.

Several factors in the development of ovarian failure could not be examined in the analysis. These include fractionation of radiation doses, time between multiple radiotherapy courses, and additive or synergistic effects of specific drugs. Although our results indicate no influence of chemotherapy, more intensive treatment regimens in current use may have greater effects on fertility and ovarian function.

In conclusion, further improvements in treating female children with cancer must be found so that the reproductive and steroidogenic capacity of the ovaries is preserved while the malignancy is cured. According to findings presented in this study, special attention must be given to means of modifying gonadal radiation exposure. The need for this special attention grows as an increasing number of children who survive for a long term will present with late sequelae, such as ovarian failure.

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# Modulation of protein expression in endometrial adenocarcinoma cells by in vitro exposure to estradiol and progesterone

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Protein maps of in vitro hormone-modulated adenocarcinoma cells from an endometrial tumor are described. It is noted that mapping of tumor tissues by means of the two-dimensional analysis described by O'Farrell and associates<sup>6</sup> of <sup>35</sup>S-methionine-labeled proteins can provide a characteristic map for each tumor and that the methionine-containing proteins of tumor cells can be independently modulated by the in vitro additions of estradiol and progesterone. Such protein modulation could be indicative of hormonal therapeutic responsiveness of individual endometrial adenocarcinomas and other hormone receptor-positive tumors. Tumor maps may further provide data for the identification and isolation of new tumor markers which might be correlated with the radiotherapeutic and chemotherapeutic sensitivity of the malignancy. (AM. J. OBSTET. GYNECOL. 139:67, 1981.)

EVALUATION of tumor responsiveness to endocrine manipulation has been carried out with some success by hormone receptor analysis. Patients with receptor-positive breast tumors have been shown to have a better prognosis than those with tumors which are borderline or negative.<sup>1-4</sup> Evidence for responsiveness of endometrial adenocarcinoma to progestin therapy has also been documented<sup>5</sup> and suggests that receptor-positive tumors are responsive to progestin therapy. Receptor positivity, therefore, would appear to be a basic re-

quirement for hormone responsiveness of a target organ or tissue; however, receptor positivity may not always result in responsiveness due to the possibility of defective binding of the hormone or defective mechanisms in processing the hormone receptor complex. It is, therefore, appropriate that estrogen or progestin efficacy be evaluated by analyzing the direct responsiveness of tumors or tissues to individual hormones. The present approach has been to develop "tumor maps" of <sup>35</sup>S-labeled proteins from the tumor cells separated by the technique of O'Farrell and associates<sup>6</sup> of two-dimensional analysis after an appropriate in vitro exposure to hormone and <sup>35</sup>S-methionine. Such maps provide a characteristic display of proteins for each tumor, indicating its metabolic and histologic make-up. It is reasonable to assume that the presence or absence of individual proteins could predispose the tumor to therapeutic responsiveness or resistance. Protein markers which might indicate such sensitivity or resistance would have significant value in the selection of therapy for individual cases. The present study provides evidence that cells from a hormone receptor-

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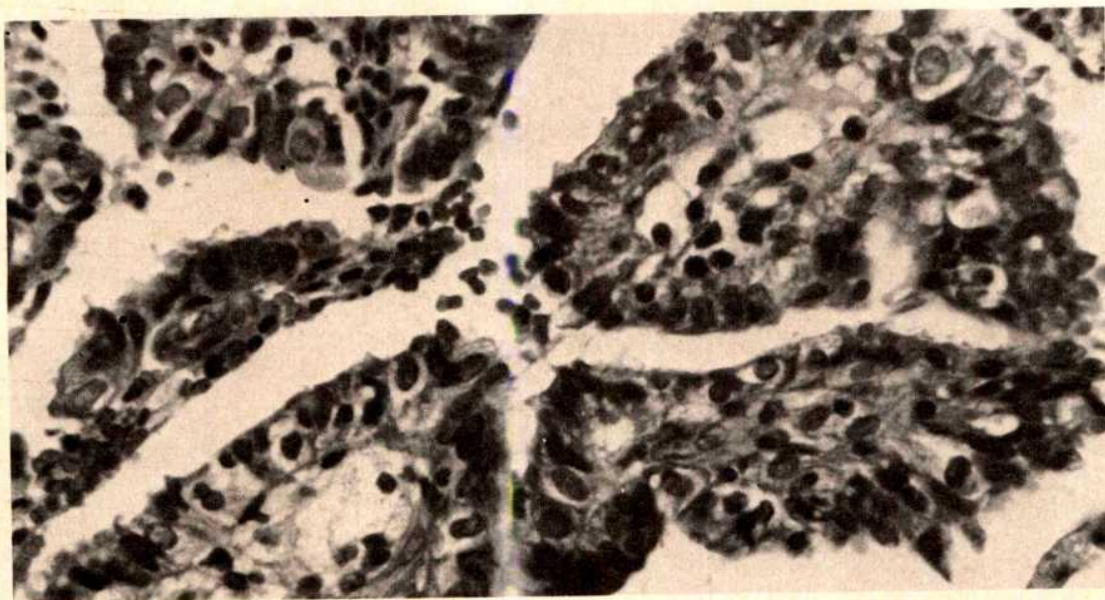


**Fig. 1.** Well-differentiated adenocarcinoma of the endometrium with papillary features. (Hematoxylin and eosin.  $\times 400$ .)

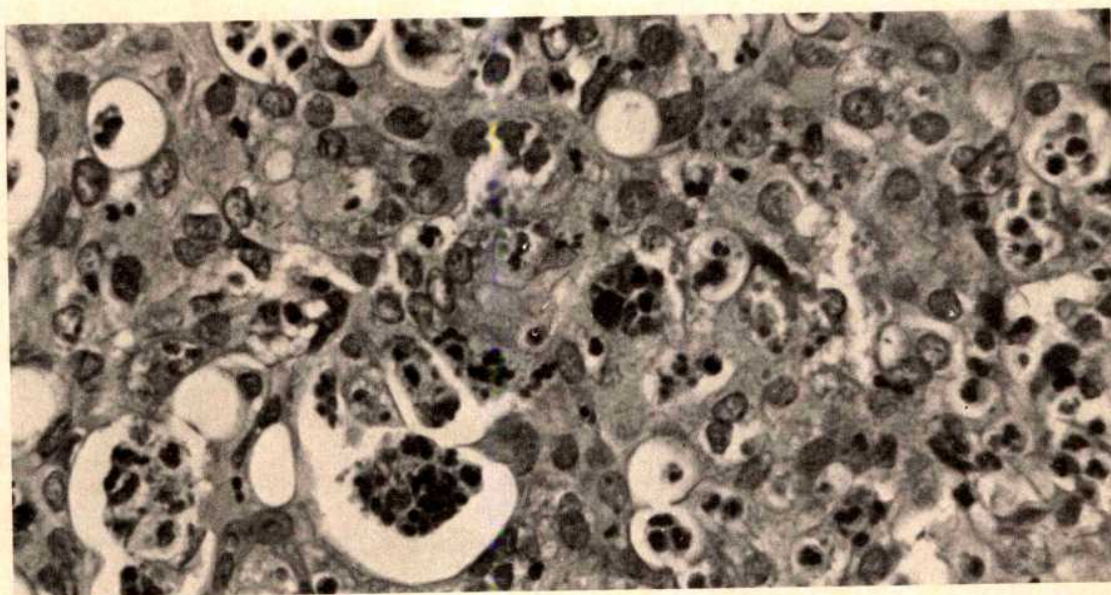


**Fig. 2.** Solid area of poorly differentiated adenocarcinoma with microcysts and infiltration by inflammatory cells. (Hematoxylin and eosin.  $\times 400$ .)

positive endometrial adenocarcinoma as shown by the tumor maps respond in vitro to the presence of estradiol and progesterone.

#### Material and methods

**Tissue collection and processing.** The tumor tissue used in the present study was from a 62-year-old Caucasian woman, gravida 1, para 1, who had been menopausal for 20 years and presented with a 3-month

history of intermittent vaginal spotting and passage of occasional clots. The patient was hospitalized, and a curettage was performed. The pathology report on the curettings was grade 3 carcinoma of the endometrium and endocervix. The patient was referred to the Southern California Cancer Center where the diagnosis was confirmed. The patient received 4,000 rads of irradiation to the whole pelvis over 5 weeks followed 2 weeks later by an intracavitary radium implant for a



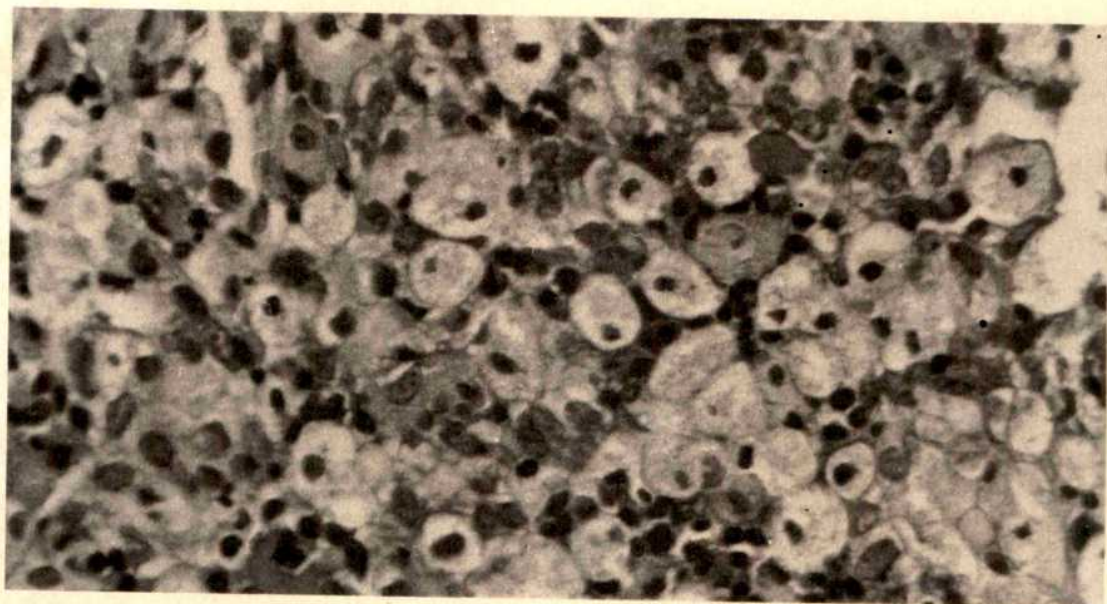


**Fig. 3.** Solid area of carcinoma with numerous lipid-laden histiocytes. (Hematoxylin and eosin,  $\times 400$ .)

central dosage of 3,000 mg hours. Three weeks later a total abdominal hysterectomy with bilateral salpingo-oophorectomy was carried out. The patient had an uneventful recovery and was placed on a regimen of megestrol acetate, 40 mg twice daily. The patient has been free of disease for the past 9 months.

At operation a portion of the tumor was taken for histologic examination and part was saved for hormone-binding studies. The pathologist reported that the tissue was grossly the equivalent of 1 ml of formalin-bathed, mahogany red, soft, irregular endometrial curettings.

Microscopically, sections showed sparse amounts of malignant endometrial glandular elements in fragmented papillary and solid form. The former was well differentiated and the latter was poorly differentiated. Foci of papillary carcinoma had minimal nondescript stroma (Fig. 1) in contrast to the solid areas (Figs. 2 and 3) wherein microcystic spaces and numerous vacuolated cells accompanied inflammatory cells. Most of the former had small, central to slightly eccentric nuclei surrounded by foamy cytoplasm most consistent with lipid-laden histiocytes.

The part of the tumor specimen for hormone-binding studies and mapping was collected directly into ice-cold phosphate-buffered saline. The specimen was sliced into 3 mm cubes, and 100 mg (about one third) was transferred to storage buffer (50 mM tromethamine, pH 7.4, 1 mM dithiothreitol, and 50% glycerol) and stored in liquid nitrogen for steroid receptor analysis. The remaining tissue was transferred to 5 ml of

methionine-deficient medium (Weymouth's medium containing 20% fetal calf serum, 1  $\mu$ M methionine, 50  $\mu$ g/ml of Kanamycin, 15 IU/ml of penicillin, and 15  $\mu$ g/ml of streptomycin) and incubated at room temperature for 30 minutes in the presence of 0.1% Collagenase II (Worthington). The dispersed cells were harvested by centrifugation at  $800 \times g$  for 10 minutes and resuspended in 2.0 ml of methionine-deficient medium. Aliquots (0.5 ml) were then placed in individual culture tubes containing 200  $\mu$ Ci of  $^{35}$ S-methionine (approximately 800 Ci/mmol, Amersham Corporation). Individual tubes were then classified as control (no additives except 5  $\mu$ l of 70% alcohol), estradiol (5  $\mu$ l of estradiol in 70% alcohol, final concentration  $10^{-8}$ M), progesterone (5  $\mu$ l of progesterone in 70% alcohol, final concentration  $10^{-8}$ M progesterone), and estradiol plus progesterone (5  $\mu$ l of a mixture of estradiol and progesterone in 70% alcohol, final concentration  $10^{-8}$ M of each steroid). The culture tubes were incubated overnight at 37° C on a rocker. Sulfur-35-labeled cells were harvested by centrifugation followed by removal of the  $^{35}$ S medium. The cells were resuspended and washed twice with 10 ml of complete Weymouth's medium. The cells were then washed with 0.35M mannitol in distilled water containing 0.01M tromethamine, pH 7.4, to remove salts. The cells were lysed in 50  $\mu$ l of buffer (9.5M urea, 2% NP<sub>40</sub>, 2% ampholytes, and 5% mercaptoethanol) by resuspension in plastic pipettes for 5 minutes at 4° C. The  $^{35}$ S lysate was assayed for radioactivity by separating aliquots of 2  $\mu$ l into 0.5 ml of 0.1% bovine serum albumin and 0.5



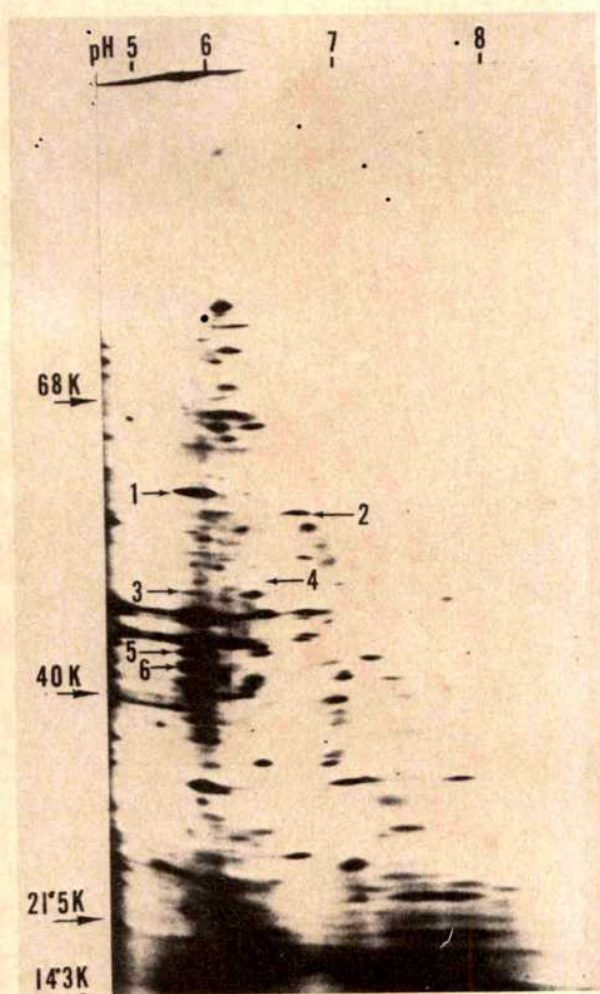


**Fig. 4A.** Figs 4A to 4D are  $^{35}\text{S}$ -protein maps of adenocarcinoma. Approximately  $0.5 \times 10^6$  cells were labeled overnight at  $37^\circ\text{C}$  in  $^{35}\text{S}$ -methionine medium.  $^{35}\text{S}$ -labeled cells were washed, pelleted, and lysed at  $4^\circ\text{C}$ . A total of  $20\ \mu\text{l}$  of lysate (200,000 cpm) was applied to each focusing gel and the samples were focused for  $5\frac{1}{2}$  hours at 400 V for 12 gels. The second dimension was composed of a 10% to 16% sodium dodecyl sulfate-polyacrylamide gradient run for approximately  $3\frac{1}{2}$  hours or until the bromophenol blue dye entered the anode buffer. The gels were dried after Coomassie Blue staining and destaining and exposed to XR2 x-ray film (Kodak) for 14 days. X-ray films were developed in an automatic x-ray developer and photographed on Kodak High Contrast Copy Film. Positive proofs were produced on medium-contrast paper. Unlabeled marker proteins used for molecular weight determination were lysozyme, trypsin inhibitor, ovalbumin, and bovine serum albumin. Fig. 4A = control; Fig. 4B = estradiol; Fig. 4C = progesterone; Fig. 4D = progesterone plus estradiol.

ml of 10% trichloroacetic acid at  $4^\circ\text{C}$ . After 20 minutes the protein precipitate was collected on a glass fiber filter (GFA) and washed with 15 ml of 5% trichloroacetic acid containing 0.1 mg/ml of methionine. The filters were dried under a heating lamp and counted in a Beckman liquid-scintillation counter.

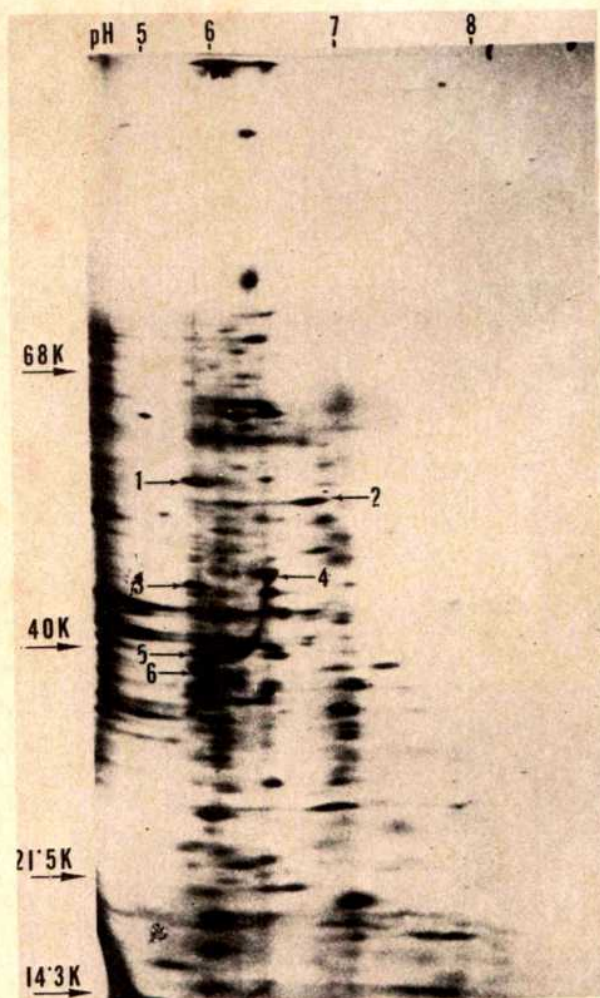


**Fig. 4B.** For legend see Fig. 4A.

**Two-dimensional analysis of  $^{35}\text{S}$  proteins.** A total of  $20\ \mu\text{l}$  of  $^{35}\text{S}$  lysate of each sample was placed on a 5% acrylamide disk gel containing 1.5% ampholytes, pH 3 to 10. The sample was overlaid with  $10\ \mu\text{l}$  of lysis buffer diluted 1:2 and focused at 400 V for  $5\frac{1}{2}$  hours, with 1% sulfuric acid and 1% ethanolamine used as the electrode solutions. After focusing, the gels were extruded from the tubes and placed in sodium dodecyl sulfate buffer (0.065M tromethamine, pH 6.8, 2.25% sodium dodecyl sulfate, 5% mercaptoethanol, and 10% glycerol) with shaking for 2 hours with one change of buffer. The sodium dodecyl sulfate-equilibrated gels were then placed on a polyacrylamide gradient slab gel (10% to 16%) and run at 20 mamp until the tracking dye (bromophenol blue) entered the lower buffer. The gels were stained with Coomassie Blue (0.1% in 50% trichloroacetic acid), destained (in 7% acetic acid), and dried. All two-dimensional procedures were essentially those of O'Farrell and associates.<sup>6</sup> Autoradiograms were obtained by exposing the gels to x-ray film (XR2,



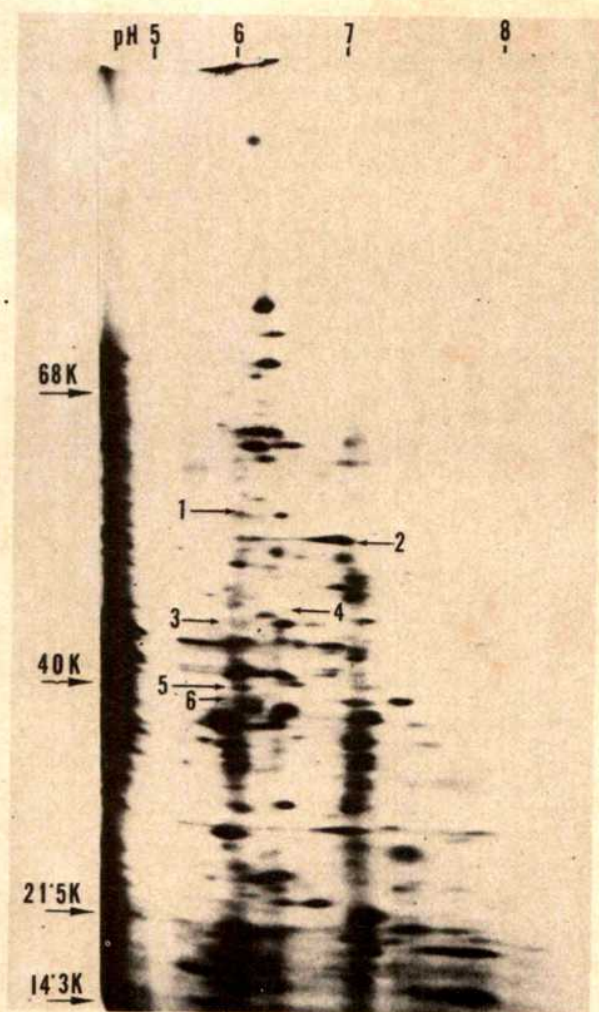


Fig. 4C. For legend see Fig. 4A.

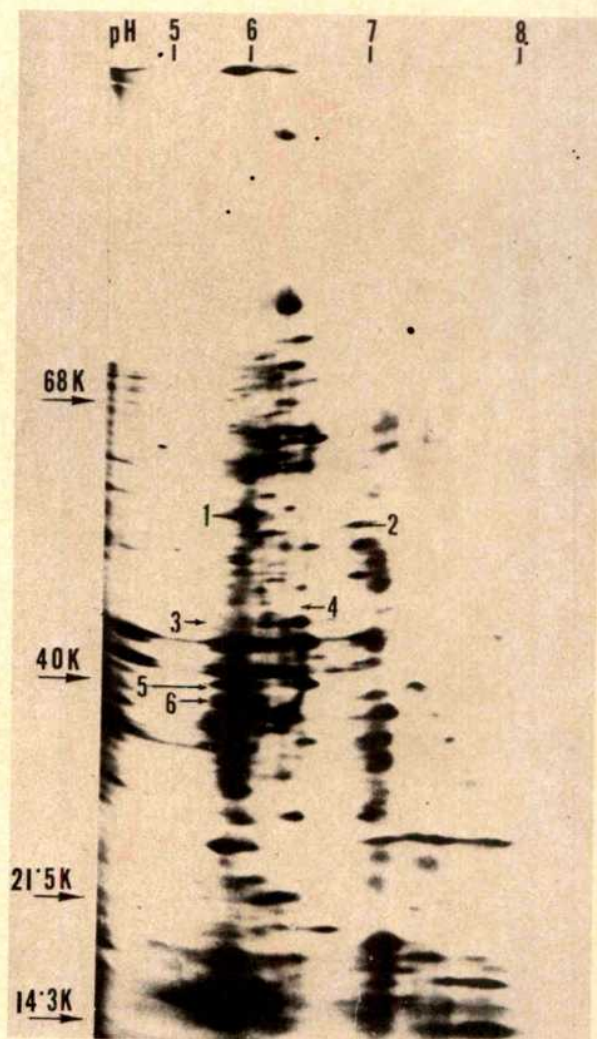


Fig. 4D. For legend see Fig. 4A.

Kodak) for approximately 2 weeks. X-ray film was developed in an automatic developer and positive pictures were made by photography of the autoradiograms with a 35 mm camera and Kodak High Contrast Copy Film followed by printing on Kodabromide Grade 3 at the original size.

**Receptor analysis.** The tissue was removed from liquid nitrogen storage (not longer than 1 week), thawed, and homogenized in 10 volumes of buffer (50 mM tromethamine, pH 7.4, 5 mM dithiothreitol 1 mM ethylenediaminetetra-acetic acid, and 10% glycerol) in 5-second bursts five times with a Tekmar Tissumizer. The homogenate was centrifuged at  $2,000 \times g$  for 20 minutes and the supernatant was recentrifuged for 1 hour at  $100,000 \times g$ . The cytosol was treated with dextran-coated charcoal for 2 minutes and recentrifuged at  $2,000 \times g$  for 10 minutes; the supernatant was diluted to 1 mg of protein per milliliter according to the Bradford<sup>7</sup> assay, and 0.2 ml (0.2 mg of protein)

was assayed for estradiol and progesterone receptors in a 200  $\mu$ l assay overnight at 4° C. Assays for progesterone receptor were carried out in 10, 2, and 0.5 nM <sup>3</sup>H-R5020 (New England Nuclear) with duplicate tubes containing 100-fold excess of unlabeled R5020. A single-point estradiol receptor assay was carried out at 10 nM <sup>3</sup>H-estradiol (tetra label, Amersham) with a duplicate tube containing 100-fold excess of diethylstilbestrol. Unbound steroid was removed by dextran-coated charcoal (5 minutes at 4° C), and the supernatants of a centrifugation at  $2,000 \times g$  (10 minutes) were counted in 20 ml of Aquasol (New England Nuclear) on a Beckman scintillation counter.

## Results

Progesterone and estradiol receptor analysis of a portion of the tumor specimen showed a positive receptor population for both hormones. The progesterone receptor concentration, based on a three-point



analysis, was found to have 177 fmoles/mg of protein while the estradiol receptor was 114 fmoles/mg of protein, based on a single-point assay. Protein maps of the in vitro hormone-modulated adenocarcinoma are shown in Fig. 4. Characteristically for this tumor, as for many other organisms and tissue which have been mapped,<sup>8-12</sup> most of the proteins have isoelectric points between the pH 5 and 7 range (Fig. 4A). On examination of all four maps it is observed that most of the <sup>35</sup>S-methionine-labeled proteins are common in all in vitro conditions. In fact, with the exceptions noted, all the major spots are common to all four maps. One of the exceptions noted was in the case of estradiol (Fig. 4B), where two new or greatly enhanced spots were present (Nos. 3 and 4). In all other maps Nos. 3 and 4 were either entirely absent or greatly reduced in quantity. The other exception was in the progesterone map (Fig. 4C), where spot No. 1 was almost completely eliminated and spots Nos. 5 and 6 were either completely gone or greatly reduced. In the progesterone plus estradiol map (Fig. 4D), the obvious spots resembled those in the control map.

#### Comment

The search for tumor markers useful for diagnosis, selection of therapy, and tumor monitoring is a diverse

and long-term task. Systematic procedures which could identify such markers have obvious value for the researcher and the clinician. The described system permits individual evaluation of the significant proteins from an individual tumor and, therefore, may allow the recognition of common denominator protein markers which reflect sensitivity to specific therapy. Ultimately individual markers might be assessed independently for convenience and rapidity. Such independent testing would require isolation and development of an assay for the individual marker. While the described mapping system presents a display of <sup>35</sup>S-methionine-containing proteins, identification of individual proteins is not immediately possible. However, some physicochemical properties of unique marker proteins can be determined, i.e., the isoelectric point and the molecular weight of the whole molecule or of its subunits if it was made up of multiple subunits. These features would allow for a direct and systematic purification scheme for such marker proteins and would provide valuable indicators for the development of new diagnostic techniques and/or methods of therapy program selection.

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# Reversal of female sterilization: Comparison of microsurgical and gross surgical techniques for tubal anastomosis

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Between 1977 and 1979, 34 consecutive, unselected patients had a reversal of tubal sterilization. The characteristics of 23 patients with more than a 6-month follow-up after end-to-end anastomosis are given, as are details of the operative procedures used. The overall intrauterine pregnancy rate was 56%, and the most reversible sterilization procedures, in terms of intrauterine pregnancies, were the Pomeroy, Uchida, and Falope ring (67%). The least reversible were unipolar tubal cautery (42%) and fimbriectomy (50%). Eleven patients had a microsurgical procedure (with the microscope) and 12 a gross surgical procedure. The use of the microscope was not associated with a significantly better pregnancy rate in cases matched for poststerilization tubal length, proximal and distal to the uterus. But insufficient numbers of tubal cautery reversals have been performed for an evaluation of the use of the microscope in this particular situation. The site of the anastomosis and the postoperative length of the tube were good predictors of intrauterine pregnancies. Five of six patients who had isthmus-isthmus anastomoses on at least one side had term vaginal deliveries. Likewise, 10 of 16 patients in whom the length of the longest tube was 6 cm or more had viable pregnancies, but only one of seven patients had a viable pregnancy with tubes of 5 cm or less. (AM. J. OBSTET. GYNECOL. 139:73, 1981.)

SINCE 1976, excellent pregnancy rates (60% to 80%) have been reported in women who have undergone surgical reversal of a previous tubal sterilization procedure.<sup>1-6</sup> Those authors who describe a delicate but gross end-to-end technique for reversing sterilization, without the use of a microscope, report 65% (range, 41% to 86%) intrauterine pregnancies,<sup>4-6</sup> and those who use the microscope report an average of 71% (range, 63% to 75%).<sup>1-3</sup> These results do not differ significantly, but those authors who advocate the use of the microscope do so on the grounds that superior magnification and lighting are responsible for their success rates. However, some authors who do not use the microscope tend to consider it to be an expensive gimmick, and others accuse those who maintain that its

use is the basis of their good results of indulging in fantasy.<sup>7</sup> Unfortunately, the preoperative tubal lengths distally and proximally are not recorded in most studies, so that it is difficult to assess, first, whether the microscope is necessary at all and, secondly, if it is of value, in which situations. Another major drawback of most studies is that there is no statement of the number of patients who were refused a reversal on technical grounds, i.e., previous tubal cautery, less than arbitrary minimum length, etc., so that no true reversibility rate can be estimated for any of the various sterilization procedures. Hopefully, once reporting of such series becomes more sophisticated, valid conclusions can be made as to the best technique for each situation.

The aim of this ongoing study at the University of California, San Francisco is (1) to determine whether there is any difference in pregnancy rates between those women who have had an end-to-end anastomosis performed (by myself) with the use of the microscope and those who have had the operation performed without higher magnification, (2) to determine the various pregnancy rates after anastomosis performed in the four major segments of the tube, and (3) to determine the pregnancy rates for various lengths of tube after anastomosis.

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**Table I.** Characteristics of patients requesting reversal of sterilization

|                                                      | Winston (1977) | Gomel (1978)  | Henderson (this study) | Thomson et al., 1978 |
|------------------------------------------------------|----------------|---------------|------------------------|----------------------|
| Mean age (range)                                     | 33<br>(22-46)  | 32<br>(19-42) | 29<br>(23-38)          | 31<br>(23-40)        |
| Age at sterilization (% <30 yr)                      | 27<br>(89)     | —<br>(89)     | 24<br>(84)             | 27<br>(—)            |
| Parity                                               | 3.1            | 2.4           | 2.9                    | 3.3                  |
| Sterilization:                                       |                |               |                        |                      |
| Interval (%)                                         | 37             | 44            | 43.5                   | 67                   |
| Puerperal (%)                                        | 27             | 47            | 43.5                   | 17                   |
| After elective abortion (%)                          | 36             | 9             | 13                     | 16                   |
| Not counseled (%)                                    | 33             | 62            | 35                     | —                    |
| Reason for request: marital<br>or partner status (%) | 79             | 63            | 74                     | 75                   |

### Patients and methods

Between January, 1977, and October, 1979, 34 patients underwent reversal of sterilization at San Francisco General Hospital on the University of California Service (UCSF). By October, 1979, 25 patients had had a follow-up of 6 months or longer. All but two patients, who had had salpingostomies to reverse bilateral fimbriectomies, had bilateral end-to-end anastomoses. Of these 23 patients, the first 12 consecutive patients underwent a gross surgical technique, without the use of a microscope, which was similar to that described by Williams.<sup>4</sup> The next 11 consecutive patients underwent an almost identical procedure, except that the microscope was used (microsurgical technique) in a way similar to that described by Winston.<sup>1</sup> All procedures were performed by myself.

The characteristics of these 23 patients are detailed in Table I. The time between sterilization and reversal was variable (mean, 7.6 years; range, 1 to 17 years). The main reason that 17 patients (74%) requested a reversal was to have more children by a new partner or a new husband, and the other six patients wanted to have more children by the same husband or partner (26%). On the average, patients had decided on a reversal 1.4 years (range, 0.5 to 4 years) prior to the actual reversal operation. Eight patients (35%) had had no formal counseling prior to the sterilization procedure, and four (17%) of these patients thought that their tubes would "untie" spontaneously after 5 years. However, only seven patients (30%) were unhappy with their decision to be sterilized immediately after the initial operation.

No patient was refused a reversal on the grounds of the previous type of sterilizing procedure (except for hysterectomy or total salpingectomy), technical difficulty, or too short a tube. Preoperative assessment included (1) a search for absolute medical and surgical contraindications to future pregnancy, (2) an effort to

obtain the original operative report describing the sterilization, (3) a determination of whether the patient was having reasonably regular ovulatory cycles by history of regular menstrual periods with premenstrual molimina or, in six patients, by the serum progesterone level on approximately Day 21 of the cycle. A hysterosalpingogram was not obtained routinely, but 13 patients had undergone hysterosalpingography (HSG) prior to their referral. Laparoscopy was performed either immediately or a few weeks prior to the reversal only in those patients who had undergone a sterilization procedure after which extensive tubal damage could be expected, i.e., unipolar cauterization of the fallopian tube.

An adequate analysis of semen (more than 20 million per milliliter, 60% motility, and 90% normal forms) or postcoital test (PCT) showing more than 20 motile sperm per high-power field was obtained from all but six male partners. In these six instances, patients wanted a reversal regardless of whether their present partner was or was not fertile. All six of these patients became pregnant.

Both gross surgical and microsurgical procedures had the following common features: (1) A Foley catheter was placed in the bladder. (2) A Pfannenstiel incision was made. (3) A small plastic wound drape was placed to exclude tubes and ovaries from abrasive abdominal packs. (4) Fine jewelers' forceps, ophthalmic and neurosurgical needle holders and other delicate microsurgical instruments were used. (5) One to 3 mm of tubal scar was removed from the proximal and distal occluded ends of the tube when the expected diameters of the tube to be anastomosed were similar (i.e., isthmus-isthmus, cornu-isthmus, and ampulla-ampulla anastomoses). However, when larger diameters were expected distally (i.e., isthmus-ampulla and cornu-ampulla anastomosis), a small hole, approximately 1.2 mm in diameter, was made in the distal occluded end



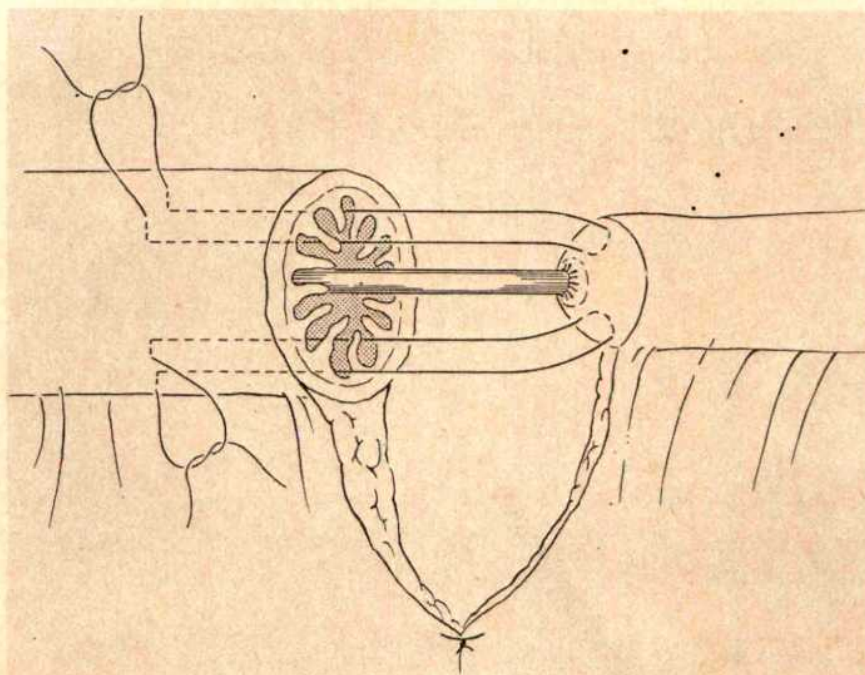


**Fig. 1.** "Hand-in-sleeve" technique. First layer: 6-0 polyethylene sutures were placed in the muscularis of the isthmus and brought out through the complete thickness of the ampullary wall, 5 to 10 mm from the cut end.

**Table II.** Outcome of 25 reversals of tubal sterilization performed between January, 1977, to October, 1979, at San Francisco General Hospital, UCSF, with a follow-up >6 months

| Pregnancy outcome              | Sterilization method |         |        |        |              |             | Totals |
|--------------------------------|----------------------|---------|--------|--------|--------------|-------------|--------|
|                                | Pomeroy              | Cautery | Uchida | Irving | Fimbriectomy | Falope ring |        |
| Term vaginal deliveries        | 6                    | 1       | 1      | —      | —            | —           | 8      |
| Third trimester                | 1                    | 1       | —      | —      | —            | 1           | 3      |
| Spontaneous abortion           | 1                    | 1       | —      | —      | 1            | —           | 3      |
| Ectopic pregnancy              | 1                    | 1       | —      | —      | —            | —           | 2      |
| Total intrauterine pregnancies | 8                    | 3       | 1      | —      | 1            | 1           | 14     |
| Total performed                | 13                   | 7       | 1      | 1      | 2            | 1           | 25     |

by passing a No. 18, 4-inch, plastic sheathed, intravenous cannula with the needle withdrawn to the occluded end of the tube and then advancing the needle.<sup>5, 8</sup> (6) A No. 2, 1 or 0 polyethylene ligature was used as a stent; this was removed either immediately after the procedure, or on the third to fifth postoperative day. When the stent was to be left in postoperatively, approximately 30 to 45 cm was fed through the proximal end of the tube into the uterine cavity, according to the technique described by Williams.<sup>4</sup> Occasionally, tubal patency was ascertained by transfundal hydrochromotubation with methylene blue, after the lower uterine segment had been occluded with a Buxton clamp. The outer surfaces of the tubes and ovaries were frequently irrigated with normal saline solution in

order to prevent drying; the pelvis was also irrigated with 6% dextran 75, just prior to closing the abdomen, and approximately 200 ml of the solution was left in the peritoneal cavity. Ampicillin, one of the cephalosporins, or penicillin and kanamycin were started intravenously during the operation, if the stent was to remain in situ after the operation. No postoperative anti-inflammatory agents, such as antihistamines and corticosteroids, and no hydrotubations were used. Stents were removed without difficulty by means of a crochet hook on the day of discharge, and the antibiotic treatment was stopped.

The gross technique involved the placing of four 6-0 polyethylene sutures, on taper point, atraumatic needles equidistant apart for the first layer of the end-to-



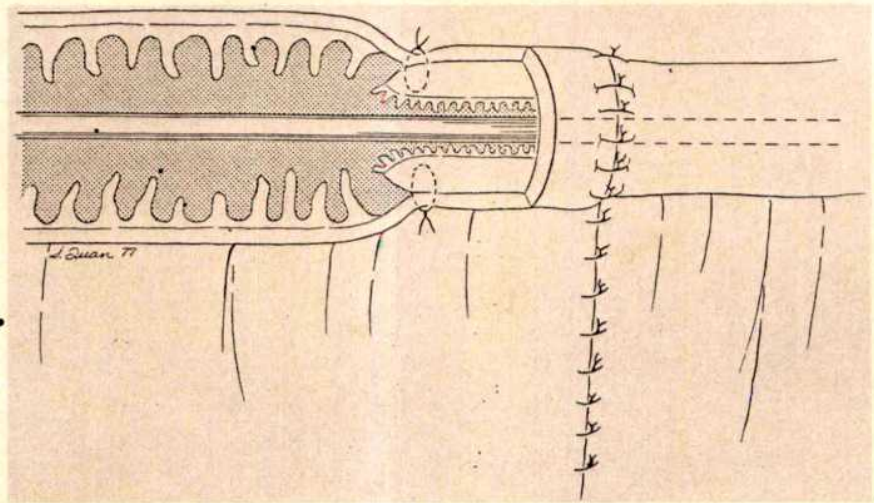


**Fig. 2.** Cutaway diagram showing end result of "hand-in-sleeve" technique. Second layer: Interrupted 6-0 polyethylene sutures placed in tubal serosa and both leaves of mesosalpinx.

**Table III.** Reversal of sterilization in patients without viable intrauterine pregnancies (N = 16), San Francisco General Hospital, UCSF, January, 1977 to October, 1979

| Pregnancy outcome        | Sterilization method |         |        |              | Totals |
|--------------------------|----------------------|---------|--------|--------------|--------|
|                          | Pomeroy              | Cautery | Irving | Fimbriectomy |        |
| Clomiphene/patent tubes  | 4 (1*)               | 1       | 1      | —            | 6      |
| Not trying/patent tubes  | 1                    | 1       | —      | 1            | 3      |
| Bilateral tubal blockage | 1 (1*)               | 1       | —      | —            | 2      |
| Lost to follow-up        | 1                    | 3       | —      | 1            | 5      |
| Totals                   | 7                    | 6       | 1      | 2            | 16     |

\*Vaginal Pomeroy procedure.

end anastomosis. The suture included just the muscularis layer in the cornual or isthmal segments of the tube, and both the muscularis and the mucosa in the ampullary segments. The serosa and the two layers of the mesosalpinx were sutured with either interrupted 4-0 or 5-0 plain or chromic catgut or 6-0 polyethylene.

When the microscope was used, 8-0 monofilament nylon sutures were used for the first layer and interrupted 6-0 polyethylene for the serosa and the mesosalpinx. Ten patients were discharged home on the fourth postoperative day (day of operation counted as day zero), nine on the third day, and four on the fifth day, and there were no complications.

Follow-up of patients was routinely performed 2 to 6 weeks postoperatively, and they were encouraged to start trying to conceive in the cycle after their first postoperative menstrual period. If patients were thought to be oligoovulatory or to have luteal phase deficiencies prior to operation, they were started on clomiphene and their response to this was monitored once a month with PCTs and serum progesterones. Patients who were not pregnant 6 months after the operation were

assessed for normal ovulatory cycles and adequate luteal phases, mainly by examination of the serum levels of progesterone, and were treated with clomiphene when necessary. When patients were shown to be having good ovulatory cycles with adequate luteal phases, their tubes were then assessed by HSG and laparoscopy.

**Results**

Table II shows the results in the 25 women who had a reversal of sterilization at this hospital. The overall intrauterine pregnancy rate was 56% (14 of 25). The most successfully reversed procedures were the Pomeroy, with 8 of 13 patients (62%) achieving intrauterine pregnancies, the Uchida procedure, with one patient who had a term vaginal delivery, and the Falope ring, with one patient who at present has a pregnancy in the third trimester. Only three of the seven patients (43%) who had a tubal cautery achieved an intrauterine pregnancy. Two patients had fimbriectomies, and one patient conceived but aborted spontaneously at 8 weeks. One patient had a ruptured ec-



topic pregnancy on the right side after the reversal of a Pomeroy procedure, and one after a tubal cauterization. The technique for the isthmus-ampulla anastomosis of the right tube in the patient with a Pomeroy reversal differed from that for all other such anastomoses, in that a "hand-in-sleeve" telescoping procedure was performed as shown in Figs. 1 to 3, with the use of interrupted 6-0 polyethylene sutures. This technique was designed to solve the problem of anastomosing tubal ends of widely different diameters and, therefore, is not recommended.

Details on the 16 patients who have not so far achieved a viable pregnancy, i.e., a term delivery or a third-trimester pregnancy, are shown in Table III. Five patients were lost to follow-up, and three patients were not trying to get pregnant, so that the corrected viable pregnancy rate for the study was 65% (11 of 17). However, for the remainder of the analysis, these 8 patients will be considered to have not become pregnant. Six patients with patent tubes, on HSG, were being treated with clomiphene to induce or augment ovulation because of anovulatory cycles or inadequate luteal phases, suggested by serum levels of progesterone determined 7 days prior to the next onset of menses. Ovulation was considered to have occurred if the serum progesterone was 5 ng/ml or more, and the luteal phase was considered to be adequate if the serum progesterone was more than 10 ng/ml.

Twenty-three patients underwent an end-to-end anastomotic technique, and 13 achieved intrauterine pregnancies (56.5%). Eleven patients who underwent the gross surgical technique were matched for site of the anastomosis (isthmus-isthmus, cornu-ampulla, etc.), the remaining length of tube distal and proximal to the uterus on both sides, age, parity, and male fertility status, with 11 patients in whom the microscope was used (Table IV). The intrauterine pregnancy rates with use of the two methods were not significantly different. When pregnancy outcome was examined in terms of type of previous sterilization procedure and microsurgical or gross surgical reversal techniques (Table V), it was seen that there was no significant difference in viable pregnancy rates (vaginal deliveries plus third-trimester pregnancies) between the gross surgical and microsurgical techniques (56% and 57%, respectively). However, there were insufficient numbers in the cautery group to adequately assess the data, but there is a suggestion that the microsurgical technique may be superior in terms of pregnancies.

The 23 patients were considered together as a group in order to assess the significance of tubal length measured to the nearest centimeter after anastomosis (Table VI), and the site of the end-to-end anastomosis

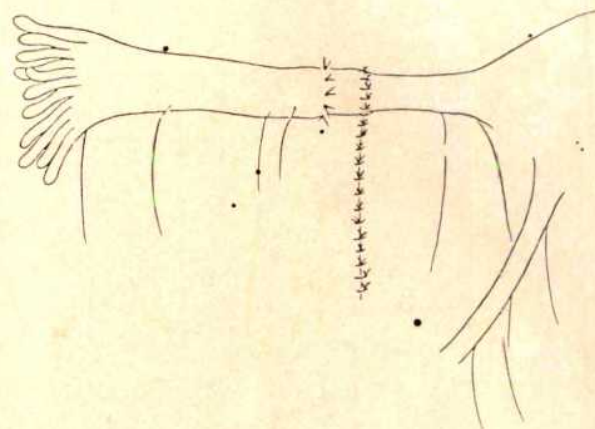


Fig. 3. End result of "hand-in-sleeve" technique.

Table IV. Microsurgical versus gross surgical end-to-end tubal anastomosis; number of patients matched for site and tubal lengths

|                                | Micro<br>surgical | Gross<br>surgical |
|--------------------------------|-------------------|-------------------|
| Term delivery                  | 1                 | 5                 |
| Third trimester                | 2                 | —                 |
| Second trimester               | 3                 | —                 |
| Spontaneous abortion           | —                 | 2                 |
| Ectopic pregnancy              | 1                 | 1                 |
| Not pregnant                   | 4                 | 3                 |
| Total intrauterine pregnancies | 6                 | 7                 |
| Total                          | 11                | 11                |

(Table VII), on subsequent pregnancy rates. The critical tubal length in terms of pregnancy success appeared to be 6 cm, and the ratio of viable pregnancy to nonpregnancy outcomes for the length of the longest tube after anastomosis was 1:6 in tubes 3 to 5 cm long, and 10:6 in tubes 6 cm or longer; and for the shortest tube of the two, 4:9 and 7:3, respectively.

The best site for the anastomosis in terms of pregnancy rate was the isthmus, since five of six patients (83%) with at least one isthmus-isthmus anastomosis had viable pregnancies; the second was the isthmus-ampulla anastomosis, with four of nine pregnancies (44%); and the third was the cornu-isthmus anastomosis, with two of six pregnancies (33%) (Table VII).

Tubal patency was established in seven of nine patients in whom HSG was performed 6 months or more postoperatively. Of the patients with bilateral tubal obstruction, one had had a Pomeroy procedure performed vaginally followed by an isthmus-fimbria and ampulla-fimbria anastomosis, and the other had had laparoscopic tubal cautery in two places bilaterally and a bilateral cornu-ampulla anastomosis with diffuse



**Table V.** Pregnancy outcome with microsurgical and gross surgical anastomotic techniques, at San Francisco General Hospital, UCSF, 1977-1979

|                                                                     | <i>Pomeroy, Uchida, Irving, and Falope ring</i> |                       | <i>Cautery</i>       |                       |
|---------------------------------------------------------------------|-------------------------------------------------|-----------------------|----------------------|-----------------------|
|                                                                     | <i>Microsurgical</i>                            | <i>Gross surgical</i> | <i>Microsurgical</i> | <i>Gross surgical</i> |
| Term deliveries and third-trimester pregnancies (N = 11)            | 4                                               | 5                     | 2                    | 0                     |
| Nonpregnant, spontaneous abortion, and ectopic pregnancies (N = 12) | 3                                               | 4                     | 3                    | 2                     |
| Totals                                                              | 7                                               | 9                     | 5                    | 2                     |

**Table VI.** Tubal length and pregnancy outcome

|                                                            | <i>Length of longest tube (cm)*</i> |      | <i>Length of shortest tube (cm)*</i> |      |
|------------------------------------------------------------|-------------------------------------|------|--------------------------------------|------|
|                                                            | 3-5                                 | 6->6 | 3-5                                  | 6->6 |
| Term deliveries and third-trimester pregnancies            | 1                                   | 10   | 4                                    | 7    |
| Nonpregnant, spontaneous abortion, and ectopic pregnancies | 6                                   | 6    | 9                                    | 3    |

\*All tubes were measured to the nearest centimeter.

chronic inflammation in the resected tubal scars when reviewed histologically.

### Comment

The characteristics of the patients in this study who requested a reversal were similar to those reported by Winston,<sup>9</sup> Gomel,<sup>10</sup> and Thomson and Templeton,<sup>11</sup> except that the latter authors reported a greater proportion of patients who had an interval procedure (see Table V). It appears possible that from this and other studies<sup>9-12</sup> that women who have their sterilizations performed post partum or after abortion are more liable to regret their decision at a later date and seek a reversal. The majority of the patients in the present study (73%) came from the lower socioeconomic groups, which agrees with the report by Thomson and Templeton.<sup>11</sup> Use of the microscope for reversal of Pomeroy, Irving, Uchida, or Falope ring procedures did not result in a greater pregnancy than the gross surgical technique. But the number of the patients who had tubal cautery was too small to give a precise answer to the question of whether the microscope offers a distinct advantage in terms of pregnancy rate over a delicate gross surgical technique. However, this study does indicate that the site of the anastomosis makes a difference to the pregnancy rates, with the isthmus proving to be far superior, and this is in agreement with the findings of other studies.<sup>3, 5, 13</sup> It also appears from this

study that the presence of the isthmus-ampulla junction is unnecessary for fertility; this conclusion is in agreement with work in animals.<sup>14</sup>

It is interesting that the only patient who had a "hand-in-sleeve" or telescoping procedure for dealing with the problem of anastomosing vastly different diameters of tube (isthmus-ampulla anastomosis) achieved an ectopic pregnancy. An ectopic pregnancy after such a procedure may be explained by the abnormal infolded epithelium at the site of a junction sutured in this manner, as found by Shahrok and associates<sup>15</sup> in cats. The finding in this study that the critical length of the tube in terms of pregnancy was 6 cm agrees with Winston's findings reported in 1977<sup>16</sup>; but most authors do not specifically quote tubal lengths at all. In the rabbit, however, more than 47% of the tube must remain distally before fertility can be anticipated.<sup>17</sup> Hopefully, future reporting will include the number of patients excluded on technical grounds, the exact site of anastomosis, and the length of tube remaining proximally and distally on both sides, so that precise data on the reversibility of different sterilization techniques and on different reversal techniques will be possible. The evidence for corpus luteal deficiency in six patients in this study supports the finding that patients requesting a tubal sterilization reversal have a midluteal serum progesterone level significantly lower than that of women with normal cycles.<sup>18</sup>

### Conclusions

This study, so far, strongly suggests that, although a delicate technique is important, a microscope is not necessary for the performance of an end-to-end anastomosis of the fallopian tube after a Pomeroy, Uchida, or Falope ring procedure. But it is possible that reversal of cautery procedures will require the use of a microscope. However, from this study and from the literature to date, end-to-end anastomosis with or without use of the microscope is the most successful method for reversing tubal sterilization. The site of the anastomosis and resultant tubal length is important in terms



Table VII. Site of anastomosis in at least one tube and pregnancy outcome

|                                                                      | <i>Isthmus-isthmus</i> | <i>Isthmus-ampulla</i> | <i>Cornu-isthmus</i> | <i>Cornu-ampulla</i> | <i>Ampulla-ampulla</i> |
|----------------------------------------------------------------------|------------------------|------------------------|----------------------|----------------------|------------------------|
| Term deliveries and third-trimester pregnancies (N = 11)             | 5                      | 4                      | 2                    | 0                    | 0                      |
| Nonpregnant, spontaneous abortions, and ectopic pregnancies (N = 12) | 1                      | 5                      | 4                    | 2                    | 0                      |

of pregnancy rates. Pregnancy resulting in term deliveries is two to three times more likely to occur when at least one tube is anastomosed in the isthmic region, and when there is a total of 6 cm or more of tube remaining. Therefore, ideally, all sterilization procedures should damage or remove only 0.5 to 2 cm of the tube in the midisthmic portion. Future reports of pregnancies after reversal of sterilization should take into account the number of patients refused reversal

on technical grounds, the site of the anastomosis, and details of tubal length, so that the reversibility of different sterilization procedures can be assessed. The overall uncorrected intrauterine pregnancy rate in this study was 56% (14 of 25); two patients had an ectopic pregnancy (8%); and no patient was refused a reversal on grounds of technical difficulty or type of tubal sterilizing procedure.

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# Intestinal operations in patients with ovarian carcinoma

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Forty-two patients with epithelial ovarian carcinoma underwent intestinal operations at the University of California, Los Angeles, and the City of Hope National Medical Center during the course of the disease. The patients were divided into three groups for purposes of this analysis. The first group had intestinal surgery during their initial laparotomy. Patients in the second group underwent intestinal operation at the time of re-exploration for recurrent disease but had no preoperative signs or symptoms of intestinal tract dysfunction, while those in the third group underwent re-exploration for symptomatic intestinal involvement by tumor. Operative complications, postoperative morbidity, and effect of the resection on disease outcome are analyzed for each group in an attempt to define the indications for intestinal resection or bypass in patients with epithelial ovarian cancer. (AM. J. OBSTET. GYNECOL. 139:80, 1981.)

EPITHELIAL OVARIAN CARCINOMA frequently spreads as multiple small implants on serosal surfaces; however, on occasion bulky pelvic disease also involves the terminal ileum, cecum, and/or rectosigmoid. Resection of these intestinal segments in the face of diffuse metastases in the upper abdomen remains controversial. Reduction of tumor volume early in the disease course can improve the response to chemotherapy or irradiation and constitutes the basis for a decision to perform an intestinal resection. The long-term failure of treatment for residual tumor and the morbidity associated with the resection counter this argument. This

dilemma is intensified in patients undergoing re-exploration for persistent or recurrent disease after chemotherapy or irradiation as subsequent therapy remains generally ineffective.

Patients with advanced ovarian carcinoma frequently develop intestinal obstruction and operation often offers the only chance to restore gastrointestinal tract function. Operative intervention is accompanied, however, by significant morbidity and mortality secondary to a large volume of tumor, prior operation, irradiation, chemotherapy, and underlying negative nitrogen balance and must be weighed against the life expectancy of the patient. In this report we analyze the experience of the Gynecologic Oncology Service at the University of California, Los Angeles, and the City of Hope National Medical Center with patients who underwent intestinal operations as treatment of primary and/or recurrent epithelial ovarian carcinoma.

## Material and methods

From 1968 to 1977, 419 patients with ovarian carcinoma were treated at the University of California, Los Angeles, and the City of Hope National Medical Center. Forty-two of these women (10%) underwent 45 operations on the intestine during the course of disease. Twelve had intestinal surgery at the initial laparotomy in an effort to reduce the bulk of disease (Group I).

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Table I. Intestinal operations (Group 1)

| Stage | Procedure                                                               | Postoperative hospital stay (days) | Survival after operation (months) |
|-------|-------------------------------------------------------------------------|------------------------------------|-----------------------------------|
| III   | Segmental ileal resection and reanastomosis                             | 10                                 | 55 (DOD)                          |
| III   | Resection of distal ileum and ileoascending colostomy                   | 13                                 | 63 (NED)                          |
| III   | Resection distal ileum and ileotransverse colostomy                     | 10                                 | 33 (DOD)                          |
| III   | Segmental ileal resection and reanastomosis; transverse loop colostomy* | 16                                 | 6 (DOD)                           |
| III   | Right colectomy, ileotransverse colostomy, sigmoid loop colostomy       | 18                                 | 13 (DOD)                          |
| III   | Transverse colon resection and reanastomosis                            | 16                                 | 15 (DOD)                          |
| III   | Left colectomy, sigmoid resection, end transverse colostomy†            | 15                                 | 20 (DOD)                          |
| III   | Sigmoid resection, end colostomy                                        | 18                                 | 18 (DOD)                          |
| III   | Sigmoid resection, end colostomy                                        | 15                                 | 8 (DOD)                           |
| IV    | Sigmoid resection, end colostomy                                        | 18                                 | 11 (DOD)                          |
| III   | Sigmoid resection, reanastomosis                                        | 14                                 | 12 (DOD)                          |
| IIC   | Sigmoid resection, reanastomosis, protecting colostomy                  | 14                                 | 2 (DOD)                           |

DOD: Died of disease; NED: no evidence of disease.

\*Wound infection.

†Hepatitis.

Table II. Intestinal operations (Group 2)

| Procedure                                                                         | Postoperative hospital stay (days) | Residual disease (cm) | Survival after operation (months) |
|-----------------------------------------------------------------------------------|------------------------------------|-----------------------|-----------------------------------|
| Segmental resection of ileum and reanastomosis                                    | 7                                  | <2                    | 10 (DOD)                          |
| Resection of distal ileum and right colon with ileotransverse colostomy (2)       | 12*                                | >2                    | 9 (DOD)                           |
|                                                                                   | 15                                 | >2                    | 5 (DOD)                           |
| Cecal resection with ileoascending colostomy; sigmoid resection and reanastomosis | 16                                 | 0                     | 19 (DOD)                          |
| Partial transverse colectomy, transverse colostomy, mucus fistula                 | 20                                 | >2                    | 6 (DOD)                           |
| Left hemicolectomy and reanastomosis                                              | 15                                 | 0                     | 2 (DOD)                           |
| Sigmoid resection and reanastomosis; protecting colostomy                         | 16†                                | 0                     | 19 (DOD)                          |
| Sigmoid resection and reanastomosis                                               | 18                                 | <2                    | 40 (DOD)                          |

DOD: Died of disease.

\*Readmitted for gastrointestinal bleeding, subsequently developed sepsis and pulmonary embolus.

†Anastomotic leak.

Eight had intestinal operations at the time of documentation and cytoreduction of recurrent tumor. These patients had no preoperative symptoms referable to the intestinal tract (Group 2). Twenty-three patients underwent exploration with a preoperative diagnosis of intestinal obstruction secondary to recurrent ovarian carcinoma with the specific intention of resection and/or diversion of the fecal stream (Group 3). Two patients in this group underwent two separate operations for obstruction while an additional patient also had resection at the initial laparotomy. All patients were analyzed with respect to operative complications and postoperative morbidity as weighed against effective palliation or prolongation of life.

Results

**Group 1.** The intestinal operations performed at initial laparotomy in these 12 patients are shown in Table

I. The indication for resection in 11 patients was direct extension of pelvic tumor to the adjacent bowel, while in one patient a transverse colon resection was performed to remove all gross tumor. All patients in this group were discharged within 18 days of operation as both immediate and late postoperative complications were infrequent (Table I). The mean survival in this group was 16 months from operation (2 to 55 months). One patient is alive without evidence of recurrence 63 months following operation and abdominal radiation.

**Group 2.** Eight patients underwent exploration for documentation of recurrent disease; the intestinal operations shown in Table II were designed to reduce maximally the tumor burden. Three of these patients had all gross tumor removed at operation. An additional two patients had residual tumor aggregates less than 2 cm in diameter while three patients had larger aggregates remaining. Those patients with minimal or



**Table III.** Operations for symptomatic intestinal obstruction (Group 3)

| Operation                                                                                     | No. of patients |
|-----------------------------------------------------------------------------------------------|-----------------|
| <i>Small bowel obstruction:</i>                                                               | 9               |
| Small bowel resection and reanastomosis                                                       |                 |
| Ileum-ileum anastomosis                                                                       |                 |
| Jejunum-jejunum anastomosis and ileoascending colostomy                                       |                 |
| Jejunotransverse colostomy                                                                    |                 |
| Ileoascending colostomy (2)                                                                   |                 |
| Ileotransverse colostomy                                                                      |                 |
| Ileo-ileo anastomosis and ileoascending colostomy                                             |                 |
| Enterotransverse colostomy                                                                    |                 |
| <i>Large bowel obstruction:</i>                                                               | 10              |
| Transverse loop colostomy (7)                                                                 |                 |
| Descending loop colostomy                                                                     |                 |
| Sigmoid loop colostomy                                                                        |                 |
| Sigmoid resection and end colostomy                                                           |                 |
| <i>Combination small and large bowel obstruction:</i>                                         | 6               |
| Ileotransverse colostomy; transverse loop colostomy                                           |                 |
| Ileo-ileo anastomosis; ileotransverse colostomy; transverse colostomy and mucus fistula       |                 |
| Ileoascending colostomy; partial transverse colectomy; transverse colostomy and mucus fistula |                 |
| Entero-entero anastomosis and entero-transverse colostomy                                     |                 |
| Small bowel resection and reanastomosis; descending colon resection and reanastomosis         |                 |
| Right hemicolectomy; resection of terminal ileum; ileotransverse colostomy; sigmoid colostomy |                 |

no gross disease had a mean survival of 18 months with only one death prior to 10 months after resection. None of the patients in the suboptimal group survived more than 9 months (mean survival 7 months). All patients in Group 2 died of disease following a mean survival of 14 months. Four patients had received chemotherapy prior to operation. Three underwent external radiotherapy to the pelvis while one had instillation of intraperitoneal chromic phosphate. Complications in this group were infrequent but were major when they occurred (Table II).

**Group 3.** Operation was performed in 23 patients for relief of intestinal obstruction. Two patients each underwent two operations for obstruction secondary to tumor (Table III). Unlike patients in Groups 1 and 2, these women underwent bowel resection and/or bypass only for palliation. The mean hospital stay was 33 days (2 to 109 days) with a mean postoperative stay of 26 days (1 to 89 days). Ten patients suffered the complications listed in Table IV, including three postoperative deaths.

Major complications were analyzed with regard to the

**Table IV.** Operative complications—Group 3

| Complication                      | No.* |
|-----------------------------------|------|
| Postoperative death               | 3    |
| Wound infection                   | 4    |
| Recurrent small bowel obstruction | 4    |
| Sepsis                            | 3    |
| Enterocutaneous fistula           | 3    |
| Wound dehiscence                  | 2    |
| Pulmonary embolus                 | 2    |
| Gastrointestinal bleeding         | 1    |

\*There were 22 complications in 10 patients.

**Table V.** Major complications versus site of obstruction

| Site of obstruction        | No. of patients | Complications* |    | Postoperative deaths |
|----------------------------|-----------------|----------------|----|----------------------|
|                            |                 | No.            | %  |                      |
| Small intestine            | 9               | 4              | 44 | 2                    |
| Large intestine            | 10              | 1              | 10 | 1                    |
| Small and large intestines | 6               | 2              | 33 | 0                    |

\*Excludes postoperative recurrent small bowel obstruction managed conservatively.

site of intestinal obstruction (Table V) and the need to perform surgery on the small intestine. Six of 15 patients (40%) who required small bowel anastomosis developed complications in contrast to only one of 10 patients (10%) in whom obstruction of the small intestine was not encountered. Four of six patients (67%) who required multiple intestinal anastomoses developed major complications (including a postoperative death) as did four of eight patients (50%) who had received radiation therapy prior to the operation for intestinal obstruction. The single patient who developed a major complication following operation for obstruction of the large intestine had received prior radiation therapy; this patient died postoperatively.

The last eight patients operated upon in this group had nutritional support via central or peripheral hyperalimentation. Seven had obstruction which involved the small intestines. No postoperative deaths or enteric fistulas occurred in this group.

### Comment

The precise role of intestinal resection in patients with disseminated ovarian carcinoma remains to be defined. The data in this report suggest that a decision to perform an intestinal resection must be made within the context of operation for primary or recurrent disease as well as the presence or absence of signs and



symptoms of intestinal obstruction. Resection at the time of initial laparotomy was accomplished in this series with acceptable morbidity. Recent studies confirm an improved response to subsequently administered chemotherapy or irradiation in patients whose disease is amenable to complete or virtually complete resection.<sup>1-3</sup> In addition, the use of a combination of chemotherapeutic agents as initial therapy for patients with ovarian carcinoma suggests further prolongation of the disease-free interval and possible increases in rates of cure over those reported for patients treated with more conventional single-alkylating agent therapy.<sup>4,5</sup>

After primary therapy, a select group of patients have recurrent tumor which can be completely resected. Patients in Group 2 who had minimal residual tumor after intestinal resection had a survival rate which is superior to that of those patients in whom larger tumor aggregates remained (18 versus 7 months). Second-line chemotherapy with standard agents generally has been ineffective, whereas recent experience with new drugs suggests improved rates of clinically meaningful response.<sup>8-10</sup> Patients undergoing exploration for recurrent tumor who have no symptoms of bowel obstruction should be considered candidates for intestinal resection if, by removal of the involved segment, the largest aggregate of residual tumor has a diameter less than 2 cm.

Most patients in Groups 2 and 3 failed treatment with either Alkeran or Adriamycin and cyclophosphamide and were then placed on a variety of combinations of chemotherapeutic agents. As more recent combinations of drugs have been reported effective in patients with recurrent disease, there is some reason to believe that the survival data reported here are worse than could be expected with these combinations.

Intestinal surgery in patients with signs and symptoms of obstruction (Group 3) was performed only to restore intestinal function. Patients were selected for operation on the basis of a complete or virtually complete obstruction and a projected survival of at least 2 months. Although morbidity and mortality in this group were high, successful palliation as defined above was achieved in 20 of 25 operations (80%). Long-term palliation, defined as survival greater than 1 year, was accomplished in four of 23 patients (17%). The data further suggest that patients with obstruction of the small intestine or obstruction of the small and large intestines have a complication rate which far exceeds those patients with obstruction only of the large intestine. Prior radiation therapy and the necessity to perform multiple anastomoses further increase the rate of complications. The preoperative and postoperative use of parenteral nutrition in this group could improve these results.

As previously stated, the role of intestinal surgery in patients with disseminated ovarian carcinoma is controversial. We would suggest resection of involved intestinal segments at the time of the primary surgery, if such a procedure can achieve "optimal surgical reduction." This can be defined as a residual tumor aggregate measuring less than 2 cm. Intestinal surgery at the time of recurrence must be individualized. Patients with a long interval between the initial treatment and recurrence are perhaps the best candidates for such an aggressive approach. Finally, palliation in the face of intestinal obstruction and progressive disease is a highly complex issue. Physicians and families together must decide what is the appropriate therapy for each individual case.

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**Inhibits uterine  
contractions in  
preterm labor\***



New

**Yutopar<sup>®</sup>** I.V. 10mg. per ml.  
oral 10mg. tablets  
(ritodrine hydrochloride)  
the vital gift of time

\*Yutopar should be used only in suitable patients  
when clearly indicated. Yutopar is contraindicated  
before the 20th week of gestation.

**Merrell**

# Yutopar<sup>®</sup>

(ritodrine hydrochloride)

## The Vital Gift of Time in Preterm Labor in Suitable Patients

A multicenter series of randomized, prospective, double-blind, controlled studies comparing ritodrine hydrochloride with either ethanol or placebo in the treatment of idiopathic preterm labor have established Yutopar as the first effective tocolytic with generally acceptable side effects available in the U.S.<sup>†</sup> Yutopar is truly the gift of time.

|                                             | Ritodrine | Control | Significance |
|---------------------------------------------|-----------|---------|--------------|
| <b>Time in utero</b>                        |           |         |              |
| Mean, days gained                           | 32.6      | 21.3    | p<0.001      |
| Gestational Age at delivery (weeks)         | 35.5      | 34.5    | p<0.05       |
| <b>Time to mature</b>                       |           |         |              |
| Incidence of respiratory distress syndrome* | 11%       | 20%     | p<0.05       |
| <b>Time to be born<sup>†</sup></b>          |           |         |              |
| Attaining 36th week of gestation or beyond  | 52%       | 38%     | p<0.05       |
| Attaining birth weight of 2500 g or more    | 58%       | 43%     | p<0.05       |
| Incidence of neonatal death                 | 5%        | 13%     | p<0.05       |

\*When steroids were administered concomitantly to mothers to hasten fetal lung maturity, maternal pulmonary edema occurred in a few cases. (See Warnings.)

<sup>†</sup>See pregnancy statement in Precaution section of prescribing information.

# Merrell

(Please read the Product Information on the last page before prescribing Yutopar.)



# The first product indicated exclusively for the management of preterm labor in suitable patients

## Clinical Pharmacology

- **Uterine effects:**

Yutopar is a beta-sympathomimetic agent. Its preferential stimulatory effects are on beta<sub>2</sub> receptors, which result in relaxation of uterine muscle.

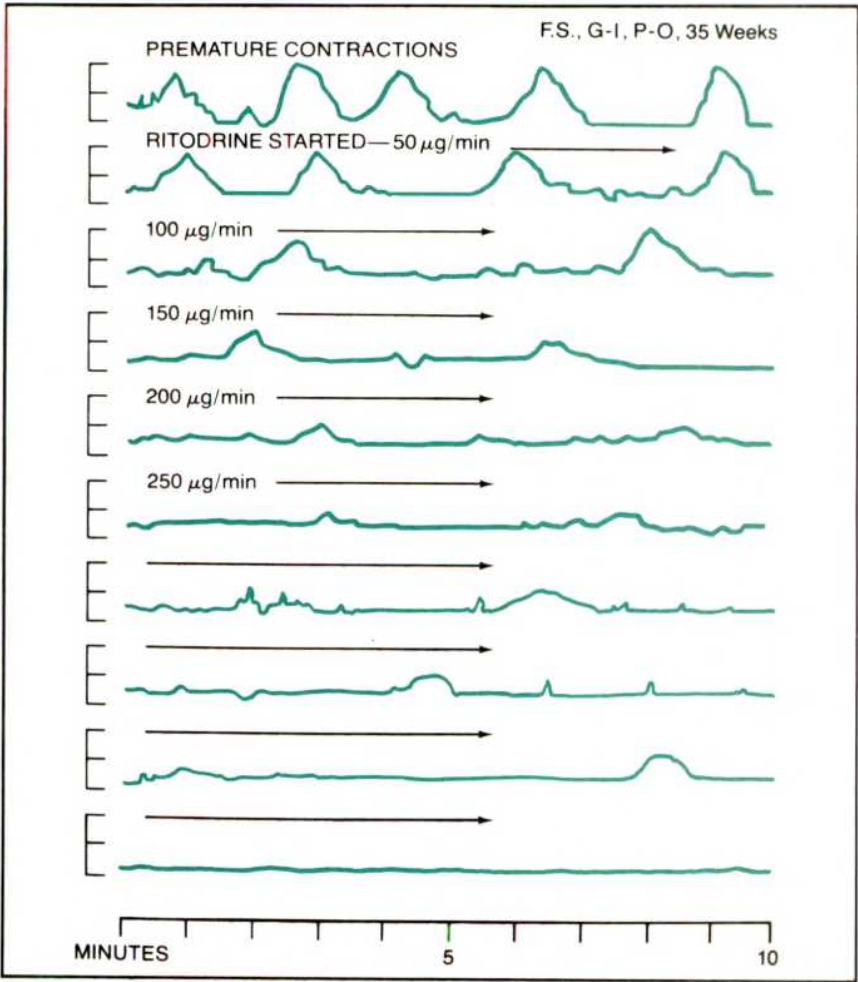


**Figure 2.** Tracing of the intravenous Yutopar infusion effect on uterine activity from a single patient in preterm labor at 35 weeks. The tracing records, in 10 minute segments monitored by external tocography, uterine activity immediately before and during a 90 minute infusion with the Yutopar dose increasing from 50 to 250 µg. per minute.

From Lauenstein *et al.*<sup>1</sup>



## • Cardiovascular effects:

The effects on the maternal cardiovascular system are dose-related.<sup>3</sup> Marked hypotension is not produced.<sup>3</sup> Cardiac output is increased.<sup>3</sup> Ritodrine passes the placental barrier in sheep<sup>4</sup> and is thought to do so in humans as well. The effects on the human fetal heart rate are modest.<sup>5,6</sup> Because cardiovascular responses are common and most pronounced during intravenous administration of Yutopar, cardiovascular effects, including maternal pulse rate and blood pressure and fetal heart rate, should be closely monitored.

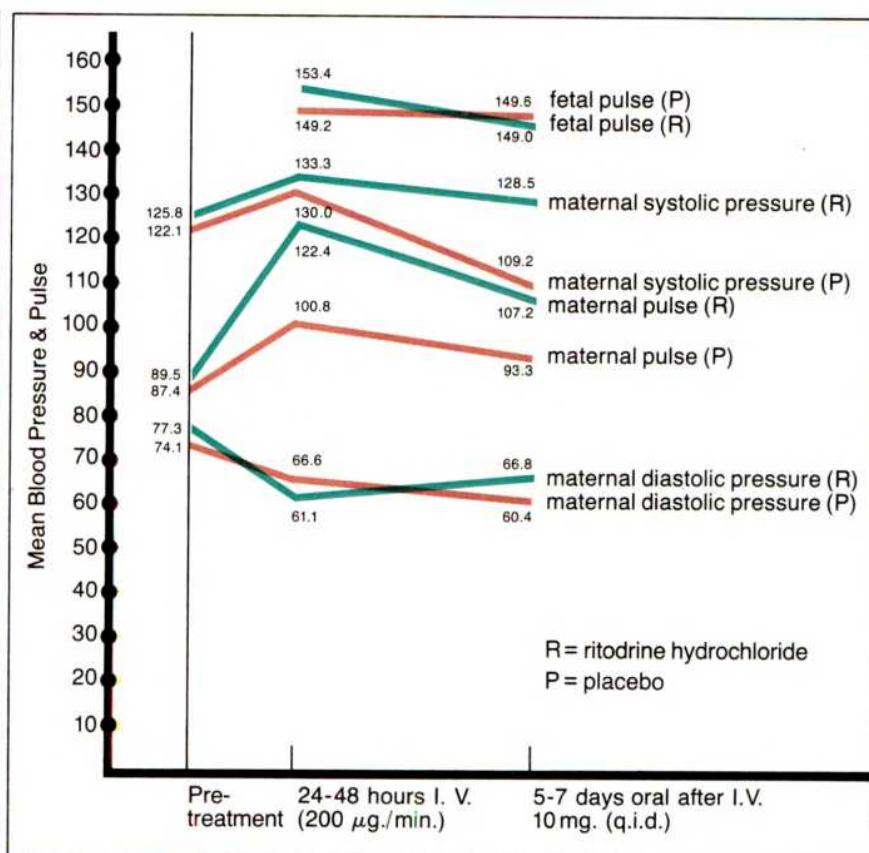
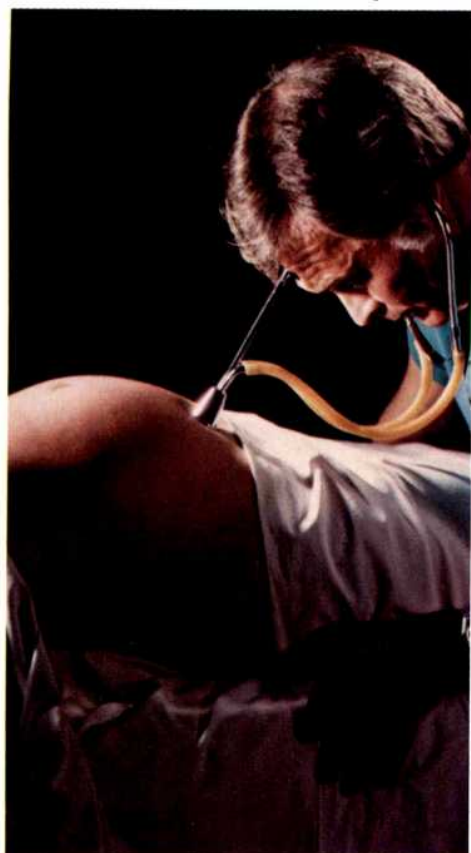


**Figure 3.** The mean maternal pulse pressure and heart rate are shown to increase during intravenous ritodrine treatment and there is a slight rise in the mean systolic blood pressure.<sup>7</sup> These effects are less marked in the control group and show a tendency to subside during oral therapy.

Based on data from Wesselijs-DeCasparis *et al.*<sup>7</sup>

## • Metabolic effects:

As a consequence of its beta-mimetic mechanism of action, ritodrine intravenously has transient metabolic effects. These may include elevations of blood glucose,<sup>8,9</sup> insulin,<sup>8,9</sup> triglycerides,<sup>10</sup> and free fatty acids.<sup>10</sup> Decreased serum iron and serum potassium have also been found.<sup>10</sup> No effects on other electrolytes have been reported.

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(Please read the Product Information on the last page before prescribing Yutopar.)



# Yutopar<sup>®</sup>

## (ritodrine hydrochloride)

### Dosage and Administration Chart

Frequent monitoring of maternal uterine contractions, heart rate, and blood pressure, and of fetal heart rate is required. The dose of Yutopar should be individually titrated according to response.

#### INTRAVENOUS

Intravenous administration of Yutopar should be supervised by persons having knowledge of the pharmacology of the drug and qualified to identify and manage complications of drug administration and pregnancy.

The patient receiving intravenous therapy should be kept in the left lateral recumbent position.

**Solution:** dilute 150 mg. ritodrine hydrochloride (3 ampuls) in 500 ml. solution, which yields a final concentration of 0.3 mg./ml. (Do not use parenteral Yutopar if the solution is discolored or contains any precipitate or particulate matter.) The following solutions may be used for dilution:

0.9% w/v sodium chloride  
5% w/v dextrose  
10% w/v Dextran 40 in  
0.9% w/v sodium chloride

10% w/v invert sugar  
Cpd sodium chloride (Ringer's)  
Hartmann's

**Method of Administration:** a controlled infusion device is recommended to adjust the rate of flow in drops per minute. An i.v. microdrip chamber 60 drops/ml. can provide a convenient range of infusion rates within the recommended dose for Yutopar.

**Dosage:** the usual initial dose is 0.1 mg./minute, to be increased according to results by 0.05 mg./minute every 10 minutes until the desired result is obtained. The effective dosage usually lies between 0.15 and 0.35 mg./minute. The infusion should generally be continued for at least 12 hours after uterine contractions cease.

| mg./min. | equals | drops/min. |
|----------|--------|------------|
| .1       | =      | 20         |
| .15      | =      | 30         |
| .2       | =      | 40         |
| .25      | =      | 50         |
| .3       | =      | 60         |
| .35      | =      | 70         |

#### Dosage equivalents

(at dilution of 3 ampuls/500 ml. fluid)  
if microdrip chamber (60 drops/ml.) is used

**Note:** At the recommended dilution and rate of administration, the maximum volume of fluid which might be administered over 12 hours at the highest dose (0.35 mg./min.) will be approximately 840 ml. The total amount of i.v. fluids administered and the rate of administration should be monitored to avoid circulatory fluid overload.

**Recurrence:** may be treated with repeated infusions, as above

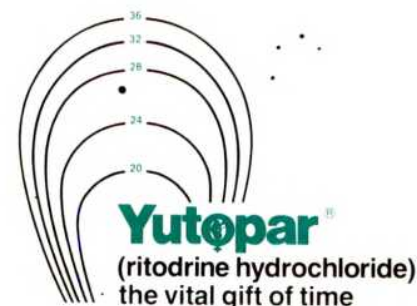
#### ORAL

**Maintenance:** 1 tablet (10 mg.) approximately 30 minutes before termination of i.v. therapy. For the first 24 hours the usual dosage is 1 tablet every 2 hours. Thereafter, the usual dosage is 1 or 2 tablets (10 to 20 mg.) every 4 to 6 hours, depending on uterine activity and side effects. Total daily dose should not exceed 120 mg.

(Please read the Product Information on the last page before administering Yutopar.)

# Merrell



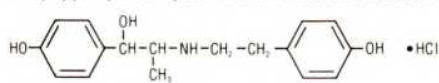


CAUTION: Federal law prohibits dispensing without prescription.

#### DESCRIPTION

Yutopar, which contains the betamimetic (beta sympathomimetic amine) ritodrine hydrochloride, is available in two dosage forms. Yutopar for parenteral (intravenous) use is a clear, colorless, sterile, aqueous solution, each milliliter contains 10 mg of ritodrine hydrochloride, 0.44% (w/v) of acetic acid, 0.24% (w/v) of sodium hydroxide, 0.1% (w/v) of sodium metabisulfite, and 0.29% (w/v) of sodium chloride in water for injection USP. Hydrochloric acid or additional sodium hydroxide is used to adjust pH. Each Yutopar tablet contains 10 mg of ritodrine hydrochloride.

Ritodrine hydrochloride is a white, odorless crystalline powder, freely soluble in water, with a melting point between 193° and 201°C. The chemical name of ritodrine hydrochloride is erythro-p-hydroxy- $\alpha$ -[1-(p-hydroxyphenethyl) amino]ethyl benzyl alcohol hydrochloride and has the chemical structure:



#### CLINICAL PHARMACOLOGY

Yutopar (ritodrine hydrochloride) is a beta-receptor agonist, which has been shown by *in vitro* and *in vivo* pharmacologic studies in animals to exert a preferential effect on the  $\beta_2$  adrenergic receptors such as those in the uterine smooth muscle. Stimulation of the  $\beta_2$  receptors inhibits contractility of the uterine smooth muscle.

In humans, intravenous infusions of 0.05 to 0.30 mg./min. or single oral doses of 10 to 20 mg. decreased the intensity and frequency of uterine contractions. These effects were antagonized by beta-blocking compounds. Intravenous administration induced an immediate dose-related elevation of heart rate with maximum mean increases between 19 and 40 beats per minute. Widening of the pulse pressure was also observed; the average increase in systolic blood pressure was 4.0 mm. Hg. and the average decrease in diastolic pressure was 12.3 mm. Hg. With oral intake, the increase in heart rate was mild and delayed.

During intravenous infusion in humans, transient elevations of blood glucose, insulin, and free fatty acids have been observed. Decreased serum potassium has also been found, but effects on other electrolytes have not been reported.

Serum kinetics in humans (non-pregnant females) of an intravenous infusion of 60 minutes duration were determined by measuring serum ritodrine levels by a radioimmunoassay technique. Three half-lives were calculated; the first of 6 to 9 minutes dominated the ascending phase of the serum drug-level curve; the second phase of 1.7 to 2.6 hours dominated the descending curve; and finally, a third phase, with a half-life of more than 10 hours, was discernible. In a study of serum kinetics after oral ingestion (male subjects), the decline of serum drug levels could be described in terms of a two-phase decay with an initial half-life of 1.3 hours and a final half-life of 12 hours. With either route of administration, 90% of the excretion was completed within 24 hours after the dose.

Comparison of ritodrine serum levels after intravenous administration with those after oral dosage indicates the oral bioavailability is about 30%. Intravenous infusion at a rate of 0.15 mg./min. for 1 hour yielded maximum serum levels ranging between 32 and 52 ng./ml. in a group of 6 non-pregnant female volunteers; maximum serum levels following single and repeated (4 x 10 mg./24 hr.) 10 mg. oral doses ranged between 5 and 15 ng./ml. and were obtained within 30 to 60 minutes after ingestion.

Placental transfer was confirmed by measurement of drug concentrations in cord blood showing that ritodrine and its conjugates reach the fetal circulation.

#### INDICATIONS AND USAGE

Yutopar is indicated for the management of preterm labor in suitable patients.

Administered intravenously, the drug will decrease uterine activity and thus prolong gestation in the majority of such patients. After intravenous Yutopar has arrested the acute episode, oral administration may help to avert relapse. Additional acute episodes may be treated by repeating the intravenous infusion. The incidence of neonatal mortality and respiratory distress syndrome increases when the normal gestation period is shortened.

Since successful inhibition of labor is more likely with early treatment, therapy with Yutopar should be instituted as soon as the diagnosis of preterm labor is established and contraindications ruled out in pregnancies of 20 or more weeks gestation. The efficacy and safety of Yutopar in advanced labor, that is, when cervical dilatation is more than 4 cm. or effacement is more than 80%, have not been established.

#### CONTRAINDICATIONS

Yutopar is contraindicated before the 20th week of pregnancy.

Yutopar is also contraindicated in those conditions of the mother or fetus in which continuation of pregnancy is hazardous; specific contraindications include:

1. Antepartum hemorrhage which demands immediate delivery
2. Eclampsia and severe preeclampsia
3. Intrauterine fetal death
4. Chorioamnionitis
5. Maternal cardiac disease
6. Pulmonary hypertension
7. Maternal hyperthyroidism
8. Uncontrolled maternal diabetes mellitus (See Precautions.)
9. Pre-existing maternal medical conditions that would be seriously affected by the known pharmacologic properties of a betamimetic drug, such as: hypovolemia, cardiac arrhythmias associated with tachycardia or digitalis intoxication, uncontrolled hypertension, pheochromocytoma, bronchial asthma already treated by betamimetics and/or steroids
10. Known hypersensitivity to any component of the product

#### WARNINGS

Maternal pulmonary edema has been reported rarely in patients treated concomitantly with Yutopar and corticosteroids and one maternal death has been reported in such a patient. Patients so treated must be closely monitored in hospital. If pulmonary edema develops, the drugs should be discontinued and the edema managed by conventional means.

Intravenous administration of Yutopar should be supervised by persons having knowledge of the pharmacology of the drug and who are qualified to identify and manage complications of drug administration and pregnancy. Because cardiovascular responses are common and more pronounced during intravenous administration of Yutopar, cardiovascular effects, including maternal pulse rate and blood pressure and fetal heart rate, should be closely monitored. Care should be exercised for maternal signs and symptoms of pulmonary edema. Occult cardiac disease may be unmasked with the use of Yutopar.

The drug should not be administered to patients with mild to moderate pre-eclampsia, hypertension, or diabetes unless the attending physician considers that the benefits clearly outweigh the risks.

#### PRECAUTIONS

When Yutopar is used for the management of preterm labor in a patient with premature rupture of the membranes, the benefits of delaying delivery should be balanced against the potential risks of development of chorioamnionitis.

Among low birth weight infants, approximately 9% may be growth retarded for gestational age. Therefore, Intra-Uterine Growth Retardation (IUGR) should be considered in the differential diagnosis of preterm labor; this is especially important when the gestational age is in doubt. The decision to continue or reinitiate the administration of Yutopar will depend on an assessment of fetal maturity. In addition to clinical parameters, other studies, such as sonography or amniocentesis, may be helpful in establishing the state of fetal maturity if it is in doubt.

#### Laboratory Tests

Because intravenous administration of Yutopar has been shown to elevate plasma insulin and glucose and to decrease plasma potassium concentrations, monitoring of glucose and electrolyte levels is recommended during protracted infusions. Special attention should be paid to biochemical variables when treating diabetic patients or those receiving potassium-depleting diuretics.

#### Drug Interactions

Corticosteroids used concomitantly may lead to pulmonary edema. (See Warnings.)

The effects of other sympathomimetic amines may be potentiated when concurrently administered and these effects may be additive. A sufficient time interval should elapse prior to administration of another sympathomimetic drug.

Beta-adrenergic blocking drugs inhibit the action of Yutopar; coadministration of these drugs should, therefore, be avoided.

With anesthetics used in surgery, the possibility that hypotensive effects may be potentiated should be considered.

#### Carcinogenesis

In rats given oral doses of 1, 10, and 150 mg./kg./day of ritodrine hydrochloride for 82 weeks, no evidence of tumorigenicity was noted.

#### Pregnancy

##### Teratogenic Effects

(Pregnancy Category B)

Reproduction studies were performed in rats and rabbits. The doses employed intravenously were 1/9 (1 mg./kg.), 1/3 (3 mg./kg.), and 1 (9 mg./kg.) times the maximum human daily intravenous dose (but given to the animals as a bolus rather than by infusion). The oral doses, 10 and 100 mg./kg., represented 5 and 50 times the maximum human daily oral maintenance dose. The results of these studies have revealed no evidence of impaired fertility or harm to the fetus due to ritodrine hydrochloride.

No adverse fetal effects were encountered when single intravenous doses of 1, 3, and 9 mg./kg./day or oral doses of 10 and 100 mg./kg./day were given to rats and rabbits on Days 6 through 15 and 6 through 18 of gestation, respectively. Intravenous doses of 1 and 8 mg./kg./day or oral doses of 10 and 100 mg./kg./day administered to the mother from Day 15 of pregnancy to Day 21 postpartum did not affect perinatal or postnatal development in rats. A slight increase in fetal weight in the rat was observed. Oral administration to both sexes did not impair fertility or reproductive performance. Lethal doses to pregnant rats did not cause immediate fetal demise. There are no adequate and well-controlled studies of Yutopar effects in pregnant women before 20 weeks gestation; therefore, this drug should not be used before the 20th week of pregnancy. Studies of Yutopar administered to pregnant women from the 20th week of gestation have not shown increased risk of fetal abnormalities. Follow-up of selected variables in a small number of children for up to 2 years has not revealed harmful effects on growth, developmental or functional maturation. Nonetheless, although clinical studies did not demonstrate a risk of permanent adverse fetal effects from Yutopar, the possibility cannot be excluded; therefore, Yutopar should be used only when clearly indicated.

Some studies indicate that infants born before 36 weeks' gestation may be less than 10% of all births but account for as many as 75% of perinatal deaths and one-half of all neurologically handicapped infants. There are data available indicating that infants born at any time prior to full term may manifest a higher incidence of neurologic or other handicaps than occurs in the total population of infants born at or after full term. In delaying or preventing preterm labor, the use of Yutopar should result in an overall increase in neonatal survival. Handicapped infants who might not have otherwise survived may survive.

#### ADVERSE REACTIONS

The unwanted effects of Yutopar are related to its betamimetic activity and usually are controlled by suitable dosage adjustment.

##### Effects Associated with Intravenous Administration

Usual effects (80-100% of patients)

Intravenous infusion of Yutopar leads almost invariably to dose-related alterations in maternal and fetal heart rates and in maternal blood pressure. During clinical studies in which the maximum infusion rate was limited to 0.35 mg./min. (one patient received 0.40 mg./min.), the maximum maternal and fetal heart rates averaged, respectively, 130 (range 60 to 180) and 164 (range 130 to 200) beats per minute. The maximum maternal systolic blood pressures averaged 126 mm. Hg (range 96 to 162 mm. Hg), an average increase of 12 mm. Hg from pretreatment levels. The minimum maternal diastolic blood pressures averaged 48 mm. Hg (range 0 to 76 mm. Hg), an average decrease of 23 mm. Hg from pretreatment levels. While the more severe effects were usually managed effectively by dosage adjustments, in less than 1% of patients, persistent maternal tachycardia or decreased diastolic blood pressure required withdrawal of the drug.

Yutopar infusion is associated with transient elevation of blood glucose and insulin, which decreases toward normal values after 48 to 72 hours despite continued infusion. Elevation of free fatty acids and cAMP has been reported. Reduction of potassium levels should be expected; other biochemical effects have not been reported.

##### Frequent effects (10-50% of patients)

Intravenous Yutopar, in about one-third of the patients, was associated with palpitation, tremor, nausea, vomiting, headache, or erythema was observed in 10 to 15% of patients.

##### Occasional effects (5-10% of patients)

Nervousness, jitteriness, restlessness, emotional upset, or anxiety was reported in 5 to 6% of patients and malaise in similar numbers.

##### Infrequent effects (1-3% of patients)

Cardiac symptoms including chest pain or tightness (rarely associated with abnormalities of ECG) and arrhythmia were reported in 1 to 2% of patients.

Other infrequently reported maternal effects included: anaphylactic shock, rash, heart murmur, epigastric distress, ileus, bloating, constipation, diarrhea, dyspnea, hyperventilation, hemolytic icterus, glycosuria, lactic acidosis, sweating, chills, drowsiness, and weakness.

##### Neonatal Effects

Infrequently reported neonatal symptoms include hypoglycemia and ileus. In addition, hypocalcemia and hypotension have been reported in neonates whose mothers were treated with other betamimetic agents.

##### Effects Associated with Oral Administration

Frequent effects (<50% of patients)

Oral ritodrine in clinical studies was often associated with small increases in maternal heart rate, but little or no effect upon either maternal systolic or diastolic blood pressure or upon fetal heart rate was found.

Oral ritodrine in 10 to 15% of patients was associated with palpitation or tremor. Nausea and jitteriness were less frequent (5 to 8%), while rash was observed in some patients (3 to 4%), and arrhythmia was infrequent (about 1%).

##### OVERDOSAGE

The symptoms of overdose are those of excessive beta-adrenergic stimulation including exaggeration of the known pharmacologic effects, the most prominent being tachycardia (maternal and fetal), palpitation, cardiac arrhythmia, hypotension, dyspnea, nervousness, tremor, nausea, and vomiting. If an excess of ritodrine tablets is ingested, gastric lavage or induction of emesis should be carried out followed by administration of activated charcoal. When symptoms of overdose occur as a result of intravenous administration, ritodrine should be discontinued, an appropriate beta-blocking agent may be used as an antidote. Ritodrine hydrochloride is dialyzable.

Acute intravenous toxicity was studied in rats and rabbits and acute oral toxicity in mice, rats, guinea pigs, and dogs. The LD50 values in the most sensitive of the species used were 64 mg./kg. intravenously in the nonpregnant rabbit and 540 mg./kg. orally in the nonpregnant mouse. The intravenous LD50 value in the pregnant rat was 85 mg./kg. The amount of drug required to produce symptoms of overdose in humans is individually variable. No reports of human mortality due to overdose have been received.

##### DOSAGE AND ADMINISTRATION

In the management of preterm labor, the initial intravenous treatment should usually be followed by oral administration. The optimum dose of Yutopar is determined by a clinical balance of uterine response and unwanted effects.

##### Intravenous Therapy

Do not use intravenous Yutopar if the solution is discolored or contains any precipitate or particulate matter.

Yutopar solution should be used promptly after preparation, but in no case after 48 hours of preparation.

**Method of Administration:** To minimize the risks of hypotension, the patient should be maintained in the left lateral position during infusion and careful attention given to her hydration.

For appropriate control and dose titration, a controlled infusion device is recommended to adjust the rate of flow in drops/minute. An i.v. microdrip chamber (60 drops/ml.) can provide a convenient range of infusion rates within the recommended dose range for Yutopar.

**Recommended Dilution:** 150 mg. ritodrine hydrochloride (3 ampuls) in 500 ml. fluid yielding a final concentration of 0.3 mg./ml. Ritodrine for intravenous infusion should be diluted with one of the following:

- 0.9% w/v sodium chloride solution
- 5% w/v dextrose solution
- 10% w/v dextran 40 in 0.9% w/v sodium chloride solution
- 10% w/v invert sugar solution
- Compound sodium chloride solution (Ringer's solution)
- Hartmann's solution

Intravenous therapy should be started as soon as possible after diagnosis. The usual initial dose is 0.1 mg./minute (0.33 ml./min., 20 drops/min. using a microdrip chamber at the recommended dilution), to be gradually increased according to the results by 0.05 mg./minute (0.17 ml./min., 10 drops/min. using a microdrip chamber at the recommended dilution) every 10 minutes until the desired result is attained. The effective dosage usually lies between 0.15 and 0.35 mg./minute (0.50 to 1.17 ml./min., 30-70 drops/min. using a microdrip chamber at the recommended dilution). The infusion should generally be continued for at least 12 hours after uterine contractions cease. With the recommended dilution, the maximum volume of fluid that might be administered after 12 hours at the highest dose (0.35 mg./min.) will be approximately 840 ml. The amount of i.v. fluids administered and the rate of administration should be monitored to avoid circulatory fluid overload. Frequent monitoring of maternal uterine contractions, heart rate, and blood pressure, and of fetal heart rate is required, with dosage individually titrated according to response.

##### Oral Maintenance

One tablet (10 mg.) may be given approximately 30 minutes before the termination of intravenous therapy. The usual dosage schedule for the first 24 hours of oral maintenance is 1 tablet (10 mg.) every two hours. Thereafter the usual dose is 1 or 2 tablets (10 to 20 mg.) every four to six hours, the dose depending on uterine activity and unwanted effects. The total daily dose of oral ritodrine should not exceed 120 mg. The treatment may be continued as long as the physician considers it desirable to prolong pregnancy.

Recurrence of unwanted preterm labor may be treated with repeated infusion of Yutopar.

##### HOW SUPPLIED

NDC 0068-0599-53: 5 ml. ampuls in boxes of 12. Each ampul contains 50 mg. (10 mg./ml.) of ritodrine hydrochloride.

NDC 0068-0595-60: 10 mg. tablets in bottles of 60. Each round, yellow tablet contains 10 mg. ritodrine hydrochloride and is inscribed YUTOPAR on one side and scored on the other.

Product Information as of July, 1980

Both the tablet and intravenous dosage forms should be stored at room temperature, preferably below 86°F. (30°C.). Protect from excessive heat.

Parenteral (intravenous) dosage form manufactured

by TAYLOR PHARMACAL COMPANY

Decatur, Illinois 62525

Distributed by

MERRELL-NATIONAL LABORATORIES

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# Merrell



## CURRENT DEVELOPMENTS

### Premenstrual syndrome

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The premenstrual syndrome (PMS) is a major clinical entity afflicting a large segment of the female population. Available information are descriptive in nature and the etiology of this syndrome remains unclear. In this review, both biochemical and psychosocial elements of the syndrome have been explored in an effort to redefine the pathophysiology of this seemingly multifactorial psychoneuroendocrine dysfunction. We propose that luteal phase sensitivity to and subsequent withdrawal from the central effects of the neuropeptides  $\beta$ -endorphin and  $\alpha$ -melanocyte-stimulating hormone result in a cascade of neuroendocrine changes within the brain-hypothalamus-pituitary complex. Modulation of neurotransmitter function by these peptides may produce alterations in mood and behavior as well as enhance pituitary release of prolactin and vasopressin. Variable gonadal steroid modulation of these responses from subject to subject likely accounts for the heterogeneous clinical manifestations of the PMS. (AM. J. OBSTET. GYNECOL. 139:85, 1981.)

ALTHOUGH RECOGNIZED for centuries by clinicians, the distressing symptoms which occur cyclically in women prior to the menses were first described by Frank<sup>1</sup> in 1931. Since then considerable effort has been directed toward the clinical characterization and pathophysiologic delineation of this premenstrual syndrome (PMS).

It has long been known that when women become irritable, tense, or depressed in the premenstrual week, a temporary deterioration in their interpersonal relationships frequently develops. Such a change has been implicated in marital discord,<sup>2</sup> baby battering,<sup>3</sup> and

criminal behavior.<sup>4</sup> With women playing an increasing role in industry, attention has been focused on the impact of PMS on absenteeism and work inefficiency. It has been observed that 36% of 1,500 women in one plant sought sedation in the premenstrual week<sup>5</sup> and estimates suggest that absenteeism due to the PMS cost a loss of 5 billion dollars in 1969 alone.<sup>6</sup> Although the association between the PMS and intellectual impairment<sup>7</sup> has not been clearly established,<sup>8</sup> there are an increased number of psychiatric admissions,<sup>9</sup> accidents,<sup>10</sup> and suicide attempts<sup>9</sup> in women during the premenstrual phase. The legal implications of PMS and the manner in which PMS-like temporary insanity may alter the legal assessment of mental competence and criminal responsibility have been the focus of a recent review.<sup>11</sup> Although crime, accident, and suicide rates are higher in women during the premenstruum it is noteworthy that these rates are still lower than those seen in the noncycling male subject.<sup>12</sup>

#### Definition and manifestations

**The classical PMS.** The patient with severe PMS develops breast swelling and tenderness, a sensation of abdominal bloating, and variable degrees of edema in the extremities late in the luteal phase. Although

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**Table I.** Common manifestations of the PMS

|                                                                     |
|---------------------------------------------------------------------|
| Headache                                                            |
| Breast swelling and tenderness                                      |
| Abdominal bloating                                                  |
| Edema of extremities                                                |
| Fatigue and depression or irritability and tension                  |
| Increase thirst or appetite and craving for sweet<br>or salty foods |
| Acneiform eruptions                                                 |
| Constipation                                                        |

true migraine headaches are uncommon,<sup>13</sup> generalized headache during the premenstruum and the first days of menses are a frequent occurrence. As the menses approach, these features are often accompanied by inward feelings of irritability and tension or fatigue and depression. An increased thirst or appetite and craving for sweets and salty foods are common. Usually relief occurs rapidly at the onset of menstruation but if menses are delayed for any reason the severity of symptoms increases.

The term premenstrual syndrome has been a point of controversy because a similar group of cyclic symptoms has been recognized on occasion at midcycle in association with ovulation<sup>6, 14, 15</sup> and less commonly in premenarchal or perimenopausal subjects<sup>16</sup> and in patients following hysterectomy with intact ovaries.<sup>17</sup> Attempts to define premenstrual tension as a subtype of the PMS in which inward feelings of anxiety predominate, serve no useful purpose and should be abandoned in favor of the accurate description of the sequence and relative severity of symptoms in patients under study. The premenstrual syndrome encompasses a variety of symptoms (Table I) and a spectrum of severity ranging from mild to incapacitating. Although to date the pathogenesis of this disorder remains speculative, the weight of evidence supports the premise that PMS is related to an aberration in the cyclic function of the hypothalamic-pituitary-ovarian axis.

**Incidence of PMS.** Efforts to compare epidemiologic data on the PMS are likely to be misleading because of variable interpretation of the clinical manifestations and the obvious difficulties encountered in quantitating the severity of symptoms. Some bias in these analyses may result from the fact that women who suffer from premenstrual symptoms are more likely to be motivated to enter studies or to answer questionnaires. The limitations of such self-report studies are inherent and have been emphasized in a controlled study by Ruble,<sup>18</sup> in which she demonstrated an increased tendency to report physical symptoms in women who were misled to believe they were premenstrual. Nevertheless, the general consensus based

on questionnaire data is that 70% to 90% of the female population will admit to recurrent premenstrual symptoms and that 20% to 40% report some degree of temporary mental or physical incapacitation.

**The association between PMS and dysmenorrhea.** PMS occurs in both ovulatory and anovulatory cycles and the incidence of this disorder correlates with neither age nor parity.<sup>9, 19-21</sup> On the contrary, dysmenorrhea is generally associated with ovulatory cycles and its incidence bears a negative correlation with increasing parity. Most authors have noted a strong clinical association between PMS and subsequent dysmenorrhea,<sup>11, 19, 22-24</sup> although reports to the contrary<sup>25</sup> leave this question unresolved.

**The effect of oral contraceptives on PMS.** Oral contraceptives are widely believed to afford relief from premenstrual symptoms. Numerous early reports suggested that premenstrual symptoms were generally fewer or less severe in oral contraceptive users than in nonusers.<sup>26-31</sup> Perhaps some of the general acceptance of this premise resulted from a failure to distinguish clearly between premenstrual symptoms and dysmenorrhea, the latter being clearly relieved by oral contraceptive therapy.<sup>32</sup> Nevertheless, 25%<sup>26</sup> to 44%<sup>33</sup> of oral contraceptive users will stop or change pills within the first year and this is not infrequently a result of intolerable side effects. This may account for reports suggesting that the incidence of premenstrual symptoms in past oral contraceptive users is higher than in present users or never-users.<sup>30, 33, 34</sup>

Others<sup>32, 35</sup> have argued that the early studies failed to take into account the striking placebo effect in this condition. Indeed tension and irritability were improved most often while somatic effects such as headache or swelling showed no change or even deterioration during oral contraceptive medication.<sup>24, 27, 33, 39</sup> In two double-blind, placebo-controlled trials dramatic improvements in premenstrual irritability and depression were reported to occur with equal frequency in both the placebo- and contraceptive steroid-treated groups.<sup>32, 35</sup>

Although a small percentage of patients with PMS may be relieved by oral contraceptive therapy,<sup>27</sup> an equal number will experience exacerbation of symptoms,<sup>26</sup> and currently there is no way to distinguish these groups before therapy is commenced. In general, oral contraceptive therapy is not a suitable treatment for PMS per se although it will often produce dramatic improvement of the dysmenorrhea which may follow.

### Pathophysiology

Numerous hypotheses have been advanced to explain the PMS but a cohesive pathophysiologic formu-



lation has yet to be established. Therapeutic dogma has been based not infrequently on anecdotal observations or the poorly controlled trials of enthusiastic investigators. This has resulted in a myriad of contradictory results, some of which are irreconcilable until further studies can be undertaken. An attempt is made in this review to examine the relative validity of existing hypotheses and to identify areas requiring clarification because of the fragmentary or inconsistent nature of available data. Recent advances in our understanding of the neuroendocrinology of the human menstrual cycle require a reinterpretation of previous data and facilitate the formulation of a new hypothesis.

**Estrogen excess or progesterone deficiency.** The data supporting the hypothesis of an excessive estrogen effect are limited and often contradictory. Frank,<sup>1</sup> in 1931, was the first to propose that PMS was due to excessive levels of female sex hormone in the blood and he recommended cathartics to eliminate excessive sex hormone through the bowel. Subsequently Israel<sup>36</sup> demonstrated proliferative endometrium in four severely affected women during the premenstruum and asserted that an unopposed estrogenic effect due to deficient progesterone production was etiologically linked to the PMS. Other reports, based on luteal phase steroid levels,<sup>37, 38</sup> endometrial biopsies,<sup>39</sup> and vaginal smears<sup>39, 40</sup> in affected women, were consistent with inadequate corpus luteal function and added support to this premise. Unopposed estrogen was felt to cause fluid retention, hyperplasia of breast tissue, and abnormal carbohydrate metabolism.<sup>39</sup> Accumulation of estrogen within the limbic system was suggested as the cause of the central nervous system manifestations of the PMS.<sup>37</sup>

In contrast, there have been numerous reports of adequate corpus luteal function in patients with PMS as determined by basal body temperature, endometrial biopsies, and assays of urinary and serum gonadal steroid levels.<sup>5, 21, 41-45</sup> These findings and the observation that symptoms were maximal as progesterone levels fell led to the inevitable conclusion that progesterone withdrawal rather than progesterone deficiency caused the PMS.<sup>25, 30</sup> While progestins are able to delay the menses temporarily the response of premenstrual symptoms to progestin therapy has been highly variable and frequently disappointing.<sup>6, 46, 47</sup> The use of exogenous progestins or testosterone to antagonize unopposed estrogen gained wide popularity in the 1950s, and, although the rationale for this therapy is cloudy, progestin administration remains popular in some centers today.<sup>48</sup> To date, there have been no large, double-blind, controlled trials demonstrating the efficacy of progestins in PMS, and until these trials have been accom-

plished and the results known the perpetuation of this therapy should be challenged.

#### **Vitamin deficiency.**

**Vitamin B.** Vitamin B therapy for the PMS had its advocates in the 1940s as it does today.<sup>50, 51</sup> However, the continued use of vitamin B<sub>6</sub> is based mainly on theoretical grounds for, despite years of clinical use, the efficacy of this treatment remains to be substantiated in controlled clinical trials. Two lines of evidence have been used to suggest a role for vitamin B<sub>6</sub> in this disorder. Initially vitamin B<sub>6</sub> was thought to correct aberrant estrogen metabolism in the PMS but more recently attention has turned to the possible role of this vitamin in the regulation of brain monoamine production.

**VITAMIN B<sub>6</sub> AND ESTROGEN METABOLISM.** In the 1940s, the Biskinds<sup>52, 53</sup> first suggested that vitamin B complex deficiency might lead to the apparent estrogen excess seen in the PMS based on their observation that vitamin B complex deficiency in rats seemed to impair estrogen metabolism. Reports of the successful treatment of PMS with vitamin B<sup>49, 52</sup> soon followed. The rationale for this treatment was questioned when Zondek and Brezezinski<sup>54</sup> demonstrated apparently normal estrogen metabolism in women with severe vitamin B deficiency during wartime and subsequently vitamin B therapy lost popularity.

**VITAMIN B<sub>6</sub> AND BRAIN MONOAMINES.** A renewed interest in vitamin B therapy occurred with the discovery that this vitamin acts as a coenzyme (pyridoxal phosphate) in the final step of biosynthesis of dopamine and serotonin. Aberrant metabolism of these brain monoamines has been implicated both in disorders of hypothalamic-pituitary function and in a variety of disorders of mood and behavior. Rose,<sup>55</sup> after an exhaustive review of the often contradictory literature relating to the interactions between vitamin B<sub>6</sub> and gonadal steroids, could only speculate that estrogen might lead to a "relative deficiency" of vitamin B<sub>6</sub> both by altering tissue distribution and by inducing hepatic enzymes which compete for available vitamin B<sub>6</sub>. Such an estrogen-induced deficiency in pyridoxal phosphate leading to a diminished biosynthesis of serotonin from tryptophan has been suggested as an etiologic factor in depression affecting both users of oral contraceptives and individuals with PMS.<sup>55-57</sup> Although in a double-blind trial significant clinical improvement followed vitamin B<sub>6</sub> administration to oral contraceptive users with depression and evidence of abnormal tryptophan metabolism,<sup>58</sup> no similar study has been carried out in the PMS.

Reports that prolactin (PRL) is elevated<sup>45, 59</sup> and that dopaminergic agents may play a therapeutic role in the PMS<sup>60</sup> led to speculation that pyridoxal phosphate

deficiency could also impair dopaminergic function in this disorder. Advocates of pyridoxine therapy for PMS believed that vitamin B<sub>6</sub> could augment dopamine biosynthesis based on initial reports that vitamin B<sub>6</sub> could depress PRL<sup>61, 62</sup> and inhibit lactation.<sup>63-65</sup> However, numerous subsequent studies involving vitamin B<sub>6</sub> administration to normal, postpartum, and hyperprolactinemic women have failed to demonstrate a significant and consistent dopaminergic effect of vitamin B<sub>6</sub><sup>66-72</sup> and have cast doubt on the rationale for vitamin B<sub>6</sub> therapy in the PMS.

**VITAMIN A DEFICIENCY.** Simkins,<sup>73</sup> in 1947, during treatment of hyperthyroidism with massive doses of vitamin A, incidentally discovered that this therapy "cured" the symptoms of premenstrual tension in one patient. Argonz and Abinzano<sup>22</sup> subsequently reported the successful treatment of 30 patients with PMS by means of large doses of vitamin A and postulated that vitamin A may be able to rectify an aberration in estrogen metabolism. Other reports on the success of vitamin A therapy in the treatment of PMS have appeared in the literature.<sup>62, 74, 75</sup> Vitamin A was thought to alleviate PMS by opposing thyroid hyperfunction<sup>76</sup> or by exerting a direct antiestrogenic<sup>74</sup> or diuretic effect<sup>74</sup> but, to date, none of these hypotheses have been substantiated.

In summary, acceptance of any vitamin deficiency theory for PMS must await further clarification of the role of vitamins in hormonal metabolism in humans, demonstration of a vitamin deficiency in affected patients when compared to control subjects, and verification of the efficacy of vitamin therapy for this syndrome in large, controlled, double-blind trials. The possibility that a cyclic vitamin deficiency exists in the PMS seems remote.

**Hypoglycemia.** As early as 1925, Okey and Robb<sup>76</sup> reported that during the menses, although fasting blood sugar levels were slightly higher, glucose tolerance test (GTT) curves were flattened and showed delayed hypoglycemia. Harris,<sup>77</sup> in 1944, observed that women frequently experienced hunger, fatigue, nervousness, sweating, and vague gastrointestinal complaints immediately prior to the menses, and an interrelationship between these symptoms of hypoglycemia and ovarian function was proposed. Billig and Spaulding<sup>78</sup> and Morton and associates<sup>79, 80</sup> independently confirmed the work of Okey and Robb.<sup>76</sup> These findings were most marked in women with prominent nervous symptoms or craving for sweets in the premenstruum. Repeat GTTs after menstruation were normal. Adequate controls were lacking in each of these studies because GTTs were not performed on a comparable series of patients without PMS.

To date, there are few reports in the literature on the effects of hormonal fluctuations on carbohydrate metabolism during the menstrual cycle. Jung and associates,<sup>81</sup> reported an overall incidence of reactive hypoglycemia (blood glucose  $\leq$  59 mg/100 ml) of 17% in a large female population and suggested that this was merely one variant of normal. However, there was no mention whether their "normal" female subjects were receiving ovulatory suppressants or hormone replacement therapy nor did they examine the relationship of reactive hypoglycemia to the phase of the menstrual cycle. Others<sup>82, 83</sup> have reported a significant decrease in oral glucose tolerance which began at midcycle and remained until the premenstruum. In contrast, significant differences were not found between follicular and luteal phase glucose tolerance and insulin response following intravenous glucose administration.<sup>84</sup> That gastrointestinal factors may profoundly influence insulin response to a carbohydrate load<sup>85, 86</sup> must be taken into account when results of oral and intravenous GTTs are compared. The recent observation by DePirro and colleagues<sup>87</sup> that there is a doubling of insulin receptor concentration on circulating monocytes in the follicular phase when compared to the luteal phase offers support to the premise that glucose tolerance may be impaired in the luteal phase with a rebound increased tolerance in the premenstruum. Such a decrease in insulin receptor concentration may be related to the development of peripheral insulin resistance similar to that found in maturity-onset diabetes.<sup>88</sup> Thus, limited evidence suggests that carbohydrate tolerance may be increased in the premenstruum and this may account for the craving for sweets experienced by some women. However reactive hypoglycemia cannot be invoked as the cause of other premenstrual symptoms because it is usually asymptomatic when it does occur<sup>81</sup> and it has been observed in normal subjects as well as in those with PMS.<sup>79, 81</sup> Further, premenstrual symptoms are rarely confined to times when hypoglycemia is likely to be maximal and are generally not relieved by ingestion of food.<sup>6</sup> Additional studies are needed to resolve the issue of changing carbohydrate tolerance during the menstrual cycle.

**Endogenous hormone allergy.** Gerber,<sup>89</sup> in 1921, and Urbach,<sup>90</sup> in 1939, were among the first to demonstrate experimentally that premenstrual urticaria resulted from hypersensitivity to some specific substance which appeared in the serum during the premenstruum. They observed that symptoms could be reproduced in these sensitive individuals, but not in normal subjects, by reinjection of serum collected premenstrually. Serum from affected patients but not from normal subjects, if administered repeatedly by



intradermal injection, would result in successful desensitization.<sup>91</sup>

Ziserman<sup>92</sup> reported the case of a 23-year-old woman with recurrent premenstrual oral and vulvar ulcers. This has since been attributed to endogenous progesterone allergy.<sup>93</sup> A variety of other skin afflictions in the premenstruum have been attributed to autoimmune progesterone dermatitis<sup>94-97</sup> and have been relieved by inhibition of ovulation<sup>94, 97</sup> or oophorectomy.<sup>97</sup> A similar dermatitis due to progesterone allergy has been reported during pregnancy.<sup>98</sup> Progesterone antibodies have been demonstrated by means of immunofluorescent techniques.<sup>94</sup> The association in women between cyclically recurring dermatitis and sex hormone allergy seems well accepted. The reason why such autoimmunity develops is uncertain although it may result from structural alteration of endogenous hormones caused by protein linkage<sup>99</sup> or from cross-sensitization by sterol-containing foods.<sup>100</sup> That such a hormone hypersensitivity can be implicated in PMS is more difficult to prove because the organs involved may be multiple and the symptoms less well defined.

Zondek and Bromberg<sup>101, 102</sup> were the first to postulate that the symptoms of PMS in select cases could be attributed to a similar hypersensitivity to endogenous hormones or their metabolites. This conclusion was based on the observation that a positive reaction to intracutaneous injection of steroids frequently occurred in women with severe PMS but not in normal control subjects. In some cases a retarded cutaneous reaction would recur at the original test site in several successive menstrual cycles. Skin reactivity to a variety of gonadal steroids and their metabolites has been reported in patients with PMS.<sup>103</sup> Several investigators attempted to desensitize patients by administering minute amounts of the steroid in question on a daily basis. Heckel<sup>103</sup> reported that 74% of his patients with PMS had a positive skin test for pregnanediol. Small amounts of this steroid were subsequently administered in three separate studies of 33,<sup>106</sup> 67,<sup>107</sup> and 288<sup>47</sup> patients, with a reported 80% overall relief from PMS. In none of these studies were there adequate controls.

The fact that many variables, including the type of vehicle, the use of adjuvants, emotional stress, and the time of testing in relation to the menstrual cycle, may profoundly affect skin reactivity<sup>103-105</sup> suggests that this alone is not enough to implicate endogenous hormone allergy in the pathogenesis of PMS. Certainly, further investigation is required to clarify the effects of gonadal steroids on human allergic response.

**Psychosomatic.** The myriad of apparently unrelated symptoms attributed to the PMS has led many authors to search for an underlying psychogenic disorder.

Israel<sup>36</sup> stated that "observing women with premenstrual tension, one cannot escape the conviction that cyclic changes in the patients' behavior must be based—in some fashion—on unconsciously expressed psychogenic reasons." He postulated that "unresolved oedipal conflicts and hidden marital discord" might underlie this neuroendocrine disorder. Suarez-Murias<sup>108</sup> believed that the patient with PMS was unconsciously utilizing her menstrual function to express distress about pressing environmental situations, difficult interpersonal relationships, and her own attitudes concerning being a woman. Others have reported a correlation between premenstrual symptoms and disturbed attitudes toward menarche and the menses,<sup>23, 109</sup> guilt feelings over sexual temptation,<sup>109</sup> severe medical and gynecologic histories,<sup>9</sup> poor sexual and marital adjustments,<sup>9, 110</sup> and high neuroticism scores.<sup>19, 24, 32, 34</sup>

Proponents of a psychosomatic basis for this disorder have reported successful treatment of PMS with the use of psychotherapy<sup>110</sup> and/or a variety of tranquilizing agents.<sup>111-113</sup> Lithium was initially reported to have beneficial effects in treatment of PMS,<sup>114</sup> but later two controlled studies<sup>115, 116</sup> did not support this finding.

Other studies supported the conflicting viewpoint that the development of the PMS frequently antedated the onset of psychiatric problems.<sup>117, 118</sup> Cyclic, predictable affective fluctuations during the menstrual cycle were found to correlate as much with cyclic endocrine changes as with core psychological characteristics.<sup>20, 119</sup>

Parker<sup>6</sup> summarized it nicely when he stated that "undoubtedly, the underlying personality pattern and the environmental setting figure importantly in the development of symptoms" but current knowledge would support the concept that "somatic factors are primary and that the psychic factors appear following the physiologic, biochemical and anatomic changes resulting from hormonal influences."

**Fluid retention.** Greenhill and Freed<sup>120</sup> were the first to postulate that sodium retention induced by ovarian steroids was the single mechanism underlying the symptoms of PMS. The predominant symptoms of any given patient were determined by the particular tissues which were most involved with fluid retention (e.g., brain—headache, gut—bloating and nausea, etc.). They believed that the cathartics which Frank<sup>1</sup> used "to eliminate excessive sex hormone" worked primarily through their dehydrating effect. A variety of diuretic agents have been employed and were alleged to relieve PMS.<sup>5, 16, 25, 112, 121-123</sup>

The large majority of these studies suffer from a common flaw—the lack of appropriate experimental design. Many claims of therapeutic success have been made without adequate placebo controls, and yet it is

well known that placebos themselves can relieve premenstrual symptoms, at least temporarily, in significant numbers of patients. Few of the studies have extended over a sufficient interval to allow for abatement of such an early placebo effect. While there are reports of no response to placebo treatment of PMS,<sup>2</sup> others have reported response rates of 15%,<sup>79</sup> 19%,<sup>124</sup> 20%,<sup>125</sup> 20% to 25%,<sup>126</sup> and up to 88%.<sup>115</sup> It is essential that future studies be performed in a double-blind fashion and that combined preparations not be used when the efficacy of a new agent is tested. In the few controlled studies performed<sup>115, 124, 127</sup> the data have failed to show improvement of premenstrual symptomatology by diuretics.

There is little doubt that some women experience significant fluid retention in the premenstruum. However, there is reason to believe that rather than causing all the other symptoms of the PMS, this generalized fluid retention is merely one manifestation of an underlying neuroendocrine disorder. The search for the factor or factors causing fluid retention in affected individuals has generated intense interest and in this review an attempt is made to summarize the voluminous literature relating to this search.

*Fluid balance during the normal menstrual cycle and in PMS.* There are few well-controlled metabolic studies on fluid balance during the normal menstrual cycle. In most cases weight gain is used as an indirect measure of fluid accumulation. The results have been contradictory. Early reports<sup>12, 128-131</sup> indicated a transient weight gain of 1 to 3 pounds in the premenstruum in 25% to 40% of women studied, with a similar transient weight gain at the presumed time of ovulation. There was often marked individual variation in weight from cycle to cycle with an equal number of women showing weight loss in the premenstruum; hence, these changes may have reflected random variation in weight. Other investigators have been unable to demonstrate any significant luteal phase weight increase.<sup>132-137</sup> In recent studies determinations of total exchangeable sodium<sup>136, 137</sup> and total body water<sup>127, 138</sup> showed no significant differences between subjects in the follicular and luteal phases. Patients receiving oral contraceptives or placebos report similar weight gains because of the medication, suggesting that on occasion psychic factors alone may contribute to weight gain.<sup>35</sup>

The literature is replete with reports of patients suffering from severe premenstrual fluid accumulation,<sup>1, 25, 120, 129, 139-142</sup> but once again there are few metabolic studies on fluid balance during the menstrual cycle in patients afflicted with PMS. The degree of premenstrual weight gain is reported to correlate

closely with the severity of premenstrual symptomatology in a few studies.<sup>5, 128, 143, 144</sup> The majority of authors have been able to establish neither a consistent pattern of significant premenstrual weight gain<sup>109, 115, 145, 146</sup> nor any evidence that a linear relationship exists between degree of fluid retention and the severity of other symptoms in those with edema in the premenstruum.<sup>15, 20, 109, 125, 134, 145</sup>

*Generalized fluid retention: One manifestation of an underlying neuroendocrine disorder.* Abdominal distention and discomfort are known to be a frequent concomitant of attacks of angioneurotic edema and are presumed to result from edema within the gut.<sup>147</sup> Edema associated with nephrosis or congestive heart failure is rarely if ever associated with these symptoms. The different pathogenesis of these disorders is well documented, and it seems that a reaction within the vascular bed of the gut leading to localized edema rather than a total body accumulation of fluid may be responsible for the symptoms of abdominal bloating, pain, and nausea.

Premenstrual mastalgia does not correlate with an increase in total body water.<sup>138</sup> During the normal menstrual cycle breast volume gradually increases by 100 ml starting just prior to ovulation and reaching a maximum on the first day of menstruation.<sup>149</sup> Premenstrual breast changes include an increase in mammary blood flow, increased water retention in connective tissue (interlobular edema), and enhanced ductal-acinar sprouting and growth. Estrogen is reported to cause histamine release from human breast tissue and animal studies suggest that this estrogen-induced histamine release may be responsible for fluid transudation, leading to interlobular edema.<sup>150</sup> However, estrogen does not produce these changes in castrate animals, affirming the complexity of the breast as a multiple endocrine target. While some authors have reported relief of mastalgia with diuretics, others have found them to be of no value.<sup>5, 25, 120</sup>

There seems to be little doubt that headache can result from excessive hydration within an indistensible cranium. Headache is a well-known concomitant of increased intracranial pressure, and there are numerous reports that severe headaches have on occasion been promptly relieved by diuresis.<sup>25, 142</sup> Ostfeld<sup>151</sup> reported some interesting experiments which cast doubt on the role of fluid retention in the causation of premenstrual headache; weight gain was induced in six patients with a history of premenstrual migraine with antidiuretic hormone or deoxycorticosterone without evoking headache, and in patients anticipating the onset of headache no relief was afforded by the induction of a prompt diuresis.



These observations support the premise that premenstrual edema is merely one manifestation of an underlying neuroendocrine disorder.

*Possible neuroendocrine causes of premenstrual edema.*

ESTROGEN, PROGESTERONE, AND THE  
RENIN-ANGIOTENSIN-ALDOSTERONE AXIS.

**ESTROGEN.** The sodium- and water-retaining effect of estrogen is reported to be slight and transient in normal women.<sup>152</sup> Estrogen increases levels of plasma renin substrate (PRS),<sup>153</sup> possibly through enhanced hepatic synthesis, which leads to elevation of plasma renin activity (PRA)<sup>153</sup> and plasma angiotensin II.<sup>154</sup> This results in an elevated secretion<sup>155, 156</sup> and excretion of aldosterone.<sup>157</sup> However, it appears that the sodium-retaining effect of estrogen is independent of its ability to augment aldosterone production,<sup>158</sup> because estradiol administration to patients with Addison's disease on a regimen of maintenance doses of adrenal steroids can still cause sodium retention.<sup>159</sup> Although little is known about the ability of estrogen to alter renal hemodynamics, this possibility must be considered.

**PROGESTERONE.** Landau and Lugibihl,<sup>160</sup> who first demonstrated a natriuretic effect of progesterone in man, postulated that progesterone was a partial agonist of aldosterone such that it could block the effect of aldosterone at the renal tubules. Recent studies on the acute and chronic effects of progesterone administration suggest that this acute natriuretic response is independent of changes in the renin-angiotensin-aldosterone axis.<sup>161</sup> Unlike estrogen, progesterone has been shown not to evoke an increase in plasma renin substrate,<sup>162, 163</sup> although it can induce an increase in PRA,<sup>163</sup> plasma renin,<sup>164</sup> and aldosterone secretion<sup>165</sup> and excretion.<sup>161, 163, 166</sup> Changes in progesterone levels are followed by parallel changes in aldosterone levels and PRA.<sup>165</sup> The fact that these changes in the renin-angiotensin-aldosterone axis occur 48 to 72 hours after progesterone administration suggests that they are a compensatory response to sodium loss.<sup>161</sup>

**RENIN-ANGIOTENSIN-ALDOSTERONE AXIS DURING THE MENSTRUAL CYCLE IN NORMAL SUBJECTS AND PMS.** During the luteal phase of the normal menstrual cycle, there is no change in the level of PRS,<sup>153</sup> but there is a rise in renin concentration<sup>150, 167</sup> leading to an increase of PRA<sup>166</sup> with an increase in plasma aldosterone levels<sup>168-170</sup> and aldosterone excretion.<sup>130, 166</sup> The fact that an increase in aldosterone occurs in the absence of an increase in PRS suggests that progesterone, not estrogen, is responsible for the rise in PRA and aldosterone during the luteal phase.<sup>166</sup> The finding that in anovulatory cycles there is a higher basal level of PRS<sup>166</sup>

with no subsequent increase in PRA<sup>166, 170</sup> or aldosterone secretion<sup>170</sup> or excretion<sup>166</sup> adds further support to this hypothesis.

Premenstrual urinary aldosterone excretion is reported to be significantly elevated in PMS patients;<sup>171-173</sup> however, plasma aldosterone levels are no different from control values.<sup>38</sup> The reports of increased aldosterone excretion await confirmation. The mechanism of any such increase is presently unclear. It may be related to altered levels of ovarian hormones, as described above, or it may simply be the result of the stress which accompanies PMS, for anxiety is known to enhance aldosterone excretion.<sup>174</sup> Recent evidence suggests that aldosterone secretion is normally under maximal tonic dopaminergic inhibition so that altered functional activity of dopamine might be implicated in enhanced aldosterone excretion.<sup>175</sup>

**VASOPRESSIN (VP).** VP is produced in the cells of the supraoptic and paraventricular nuclei and is transported as part of a neurophysin-VP complex to the median eminence and the posterior lobe where it is released upon demand.<sup>176</sup> While the fine regulation of intravascular volume and tonicity are major functions of vasopressin, the demonstration by immunocytochemistry that VP is transported to other brain regions has led to the inference that it may also modulate other brain functions.<sup>177-180</sup> Bergland and Page,<sup>181</sup> in a reappraisal of pituitary-brain-vascular system relationships, have demonstrated that some of the vessels originally thought to be adenohipophyseal portal vessels in fact represent a common capillary bed uniting the adenohipophysis and neurohipophysis. Secretion of neurophysin and VP into these "portal vessels" has been clearly established in animals.<sup>182</sup> These observations and the fact that VP can induce adrenocorticotrophic hormone release in humans<sup>183</sup> are consistent with the concept that VP may modulate anterior pituitary function. The delivery of VP to other brain areas by direct axonal transport may be important, for VP and its analogues have recently been shown to affect memory processes dramatically in animals<sup>184</sup> and man.<sup>185</sup> The physiologic significance of these observations and the possible multiple sites of action of VP (brain, anterior pituitary, etc.) remain to be elucidated.

**VASOPRESSIN IN THE NORMAL MENSTRUAL CYCLE AND IN THE PMS.** To date changes in VP levels during the menstrual cycle have not been reported. Miller and Wilks<sup>186</sup> suggested that sex steroid hormones had little effect on VP levels. In the rhesus monkey urinary VP levels in preovulatory and postovulatory periods and in pregnant and nonpregnant states were not different. Robertson<sup>187</sup> has cautioned that the rate of urinary

excretion of VP may not accurately reflect VP secretion. In rats, both pituitary content and plasma levels of VP fluctuate during the estrous cycle with peak values on the morning of proestrus when circulating estrogen levels are highest.<sup>188, 189</sup> Castration causes a decrease in plasma VP levels and estrogen therapy has the opposite effect.<sup>189</sup> Progesterone has been shown to inhibit reflex oxytocin release,<sup>190</sup> but whether it exerts a similar effect on VP is unknown.

The nature of the dopaminergic influence on VP release is not resolved. An inhibitory role for dopamine (DA) has been suggested by the report of Wolny and associates.<sup>191</sup> In rats, intraventricular injection of DA produced a diuresis which could be blocked by pretreatment with a DA receptor blocker. Other indirect evidence supports an inhibitory role of DA on VP release; ethanol which is known to increase dopaminergic activity in the brain<sup>192, 193</sup> is also known to inhibit VP release and the suspected dopaminergic hyperfunction in anorexia nervosa<sup>194</sup> is frequently associated with partial diabetes insipidus.<sup>195</sup>

In contrast, Bridges and associates<sup>196</sup> have reported that physiologic levels of DA in the *in vitro* superfused rat hypothalamic preparation caused dramatic release of VP. The intraventricular injection of DA elicited a similar response which could be blocked by low doses of the dopaminergic blockers haloperidol and pimozide. Although the reason for these conflicting results is uncertain, it may relate to differing experimental conditions and/or to the dosages of DA used.

That estrogen may regulate VP release by altering dopaminergic function is suggested by several lines of indirect evidence. Estradiol receptors are located in cells of the arcuate nucleus where the tuberoinfundibular dopaminergic neurons originate, and labeled estradiol has been shown to accumulate in the cell bodies of these neurons.<sup>197</sup> Castration is known to decrease DA turnover in this dopaminergic system while estrogen therapy has the opposite effect.<sup>198</sup>

Taken together, these observations suggest that changing estrogen levels during the menstrual cycle may modulate release of VP through induced changes in tuberoinfundibular dopaminergic activity, although this does not exclude other possible sites of estrogen action.<sup>199</sup>

VP levels have not been studied in patients with severe premenstrual edema. However, in 1940, Edeiken and Griffith<sup>141</sup> reported the presence of a bioassayable antidiuretic substance in the plasma of a patient with mitral stenosis who experienced premenstrual recurrence of pulmonary edema in 17 successive cycles. Irradiation to the pituitary region had no effect on menses but eliminated the antidiuretic principle with

concomitant relief of symptoms. Apparently the irradiation had damaged the hypothalamic magnocellular neurons responsible for VP synthesis and release without significantly disrupting anterior pituitary function, thus providing a strong clue that VP was the etiologic factor. Others have reported relief of PMS by the administration of VP antagonists<sup>5, 16</sup> but adequate controlled trials are lacking.

Valid conclusions regarding the role of VP in the PMS must await further investigation of VP levels in this condition and in normal cycles by means of a sensitive radioimmunoassay. With growing evidence that the role of VP extends beyond its ability to modulate fluid balance and that its release may be enhanced by emotional factors and sex steroids, rigorous efforts should be made to clarify the role of VP in the PMS.

**PROLACTIN.** The role of PRL in the human has been an area of intensive research, and the development of DA agonists in recent years has given further impetus to this area of study. The myriad of investigations on the neuroendocrine regulation of PRL synthesis and release and its biological function in the human have been detailed elsewhere.<sup>200</sup>

**PROLACTIN IN THE NORMAL MENSTRUAL CYCLE AND IN PMS.** Plasma PRL concentrations are reported to show a circadian periodicity with peak levels during sleep,<sup>201, 202</sup> marked individual fluctuation from day to day,<sup>201, 203</sup> a peak PRL level about the time of ovulation,<sup>165, 201, 203, 204</sup> and higher mean PRL levels in the luteal phase than in the follicular phase.<sup>165, 203-205</sup>

The possibility that PRL plays a role in the PMS was first suggested by Horrobin in 1971. Soon after there appeared several reports documenting elevated luteal phase PRL levels in PMS patients.<sup>45, 59, 206</sup> Subsequent studies have failed to confirm this finding.<sup>60, 207, 208</sup> Moreover, several controlled clinical trials of bromocriptine for this disorder have produced contradictory results; suppression of PRL secretion with bromocriptine is reported to have had a beneficial,<sup>60</sup> equivocal,<sup>45, 127, 208, 209</sup> and negative<sup>207, 210</sup> response in PMS.

The assumption that suppression of PRL will correct fluid retention presupposes that PRL is responsible for this symptom. Despite an apparent osmoregulatory role for PRL in both fish and animals, no similar effect of PRL has been clearly established in humans. Initial human studies suggesting that PRL might play an important role in fluid balance<sup>211-215</sup> have been discredited by the demonstration of VP contamination of ovine PRL,<sup>216-218</sup> the inability to reproduce osmotically induced changes in PRL levels under controlled conditions,<sup>219-222</sup> and the lack of acute osmotic changes after hyperprolactinemia induced by thyrotropin-releasing factor.<sup>219, 221, 223</sup> Isolated reports of decreased fluid re-



tention in PMS after bromocriptine therapy<sup>206</sup> must be viewed circumspectly for controlled trials have not shown this agent to be more efficacious than placebo for treatment of edema in PMS.<sup>208, 209</sup> It is possible that bromocriptine functions as a dopamine agonist within the central nervous system and at the level of the kidney to improve mood or reverse fluid retention by correcting deficient dopaminergic function at these sites. Our current understanding does not allow the conclusion that a relative elevation of prolactin per se is an important factor in cases of premenstrual edema.

**ANGIOTENSIN II.** As far back as 1926, Petersen and Milles<sup>224</sup> demonstrated an increase in capillary permeability in the premenstruum. Subsequent observations led to the conclusion that angiotensin II was the agent responsible for this generalized effect.<sup>225, 226</sup> Although many substances are capable of altering capillary permeability, angiotensin seems to exert a more pronounced effect on the mesenteric vascular bed.<sup>227</sup>

Both estrogen and progesterone can induce an increase in angiotensin II levels. Edwards and Bayless<sup>228</sup> have reported an enhanced retention of sodium and water when women were tilted from a supine to an upright position only during the luteal phase of the cycle. They proposed that the increased hydrostatic pressure caused a maximal transcapillary fluid escape when capillary permeability was increased in the luteal phase.

In PMS subjects the degree of luteal phase fluid transudation across the capillaries is reported to be significantly greater than in healthy control subjects.<sup>229</sup> Others have reported that the degree of capillary permeability correlates poorly with the severity of the PMS.<sup>230</sup> Whether the increased capillary transudation is important in the development of edema and abdominal bloating in this disorder is presently speculative.

There is now convincing evidence to suggest that angiotensin participates in the central mechanisms governing control of thirst.<sup>231</sup> At physiologic levels, angiotensin will stimulate thirst in dogs<sup>232</sup> and prolonged infusions of this agent will cause water-loaded rats to continue drinking until they become grossly edematous.<sup>231</sup> Intraventricular injection of angiotensin blockers will abolish these responses.<sup>233</sup> Dopaminergic pathways appear to play a central role in the mediation of angiotensin II-induced drinking and may also play a permissive role in normal control of thirst.<sup>234</sup> Dopaminergic agonists increase and dopaminergic antagonists attenuate water intake in animals in response to angiotensin or water deprivation.<sup>234</sup> This drinking behavior varies during the estrous cycle, being least at estrus and maximal at diestrus and metestrus. Prepubertal and ovariectomized adult rats show no cyclic

changes in this phenomenon.<sup>235</sup> Exogenous estrogens have been shown to reduce dramatically the drinking response to angiotensin II in rats.<sup>236</sup> Estrogen may exert this effect by altering dopaminergic transmission and/or dopamine turnover.<sup>198</sup>

The role of angiotensin in the PMS is uncertain although it may contribute to the development of edema or gut bloating by enhanced capillary permeability and to central stimulation of thirst. The development of the specific angiotensin II receptor blocker saralasin should allow the more precise delineation of the role, if any, of angiotensin in this disorder.

#### CATECHOLAMINES.

**NOREPINEPHRINE AND EPINEPHRINE.** There is a growing body of evidence which implicates catecholamines in the regulation of salt and water balance. Their effect on this process is complex and probably represents the summation of numerous catecholaminergic responses throughout the body. An increase in sympathetic activity may alter renal function not only by the direct effects of  $\alpha$ - or  $\beta$ -adrenergic stimulation on cardiovascular function and renal hemodynamics but also by the indirect effects of adrenergic innervation of renin-secreting juxtaglomerular cells.<sup>237</sup> These effects combine to cause an increase in renin and angiotensin. In addition, sympathetic stimulation is reported to cause VP release both by altering baroreceptor tone<sup>238</sup> and by a central mechanism in response to increasing systemic angiotensin levels.<sup>239</sup>

An interesting parallel has been demonstrated between subjects treated with the  $\beta$ -adrenergic stimulant isoproterenol and patients with idiopathic edema. Both show a diminished renal clearance of cyclic adenosine monophosphate (c-AMP) and elevated plasma c-AMP levels.<sup>240</sup> Kuchel and associates<sup>240</sup> have suggested that excessive  $\beta$ -adrenergic tone might play a role in this form of intermittent edema in the female population. Indeed, they have reported preliminary observations suggesting a useful therapeutic role for  $\beta$ -blockade with propranolol in certain patients with idiopathic edema.<sup>241</sup> No similar studies have been performed on women with PMS.

**DOPAMINE.** Since Goldberg and associates,<sup>242</sup> in 1963, demonstrated a natriuretic effect of DA in four patients with congestive heart failure, many studies have been performed in an effort to delineate further the role of this catecholamine in the regulation of sodium and water balance. It was originally demonstrated that in low-dose infusions DA increased cardiac output without affecting blood pressure and that this was accompanied by an increase in renal plasma flow (RPF), glomerular filtration rate (GFR), and a natriuresis.<sup>234</sup> Subsequently DA was shown to cause a natriuresis in

the absence of alterations in total RPF, and a redistribution of intrarenal blood flow as well as a direct inhibition of tubular sodium transport has been implicated.<sup>244</sup> The cardiovascular and renal actions of DA have been recently reviewed.<sup>244, 245</sup>

Volume expansion with saline but not with albumin is associated with an increased secretion of urinary free DA and a decreased excretion of urinary norepinephrine.<sup>244</sup> The urinary free DA/NE ratio was shown to have significant positive correlation with urinary sodium excretion in normal subjects during postural change<sup>246</sup> and during oral or intravenous salt-loading experiments.<sup>247</sup> Although the increase in urinary free DA appears to be a specific effect associated with sodium loading, the renal handling of this catecholamine has not been fully elucidated. Experimental evidence suggests that the increased urinary free DA results from tubular secretion of renal DA as a result of sodium loading.<sup>244, 247-249</sup> Impairment of conversion of L-dopa to DA after treatment with the dopa-decarboxylase inhibitor carbidopa has been shown to cause a significant fall in both urinary DA and urinary sodium excretion in man.<sup>250</sup>

**THE RENAL DOPAMINERGIC SYSTEM IN IDIOPATHIC EDEMA AND PMS.** Recently patients with idiopathic edema were shown to have lower urinary excretion of both DA and sodium than control subjects in response to upright posture and furosemide administration.<sup>241, 251</sup> Ferris and associates<sup>252</sup> observed that patients with idiopathic edema on high-salt diets failed to escape sodium retention in the normal fashion and speculated that some "third factor" regulated this response in normal individuals. Subsequently a defect in renal dopaminergic mechanisms has been suspected as an etiologic factor in idiopathic edema,<sup>240, 253</sup> and in the PMS a more generalized defect in dopaminergic function has been postulated.<sup>244</sup> In preliminary reports both L-dopa<sup>253</sup> and bromocriptine<sup>254</sup> have caused improvement in idiopathic edema. Some authors have reported success with bromocriptine for the edema in PMS<sup>60, 206</sup> but there is no uniform agreement on this finding.<sup>208, 209</sup>

Although suggestive, these findings do not allow the firm conclusion that a cause-effect relationship between a low urinary DA/NE ratio and a low urinary sodium excretion exists. It is possible that these findings are the result of some other defect in renal sodium handling. The answer to this question must await further delineation of the renal catecholaminergic mechanisms in both normal subjects and those with PMS.

**Summary.** The available data do not lend strong support to the hypothesis that all the symptoms of premenstrual syndrome result from a generalized fluid re-

tention. The poor correlation between weight gain and symptomatology and the frequent failure of symptoms to improve with diuresis alone suggest that symptoms such as headache, abdominal bloating, and breast tenderness may be the result of localized processes at these sites.

There are, however, well-documented cases of severe premenstrual edema which can be attributed to little other than a generalized fluid retention. The precise mechanism for this fluid retention remains uncertain.

Estrogen appears to be able to induce sodium and water retention both by increasing aldosterone levels and by some other unrelated mechanism that remains poorly understood. It does not appear that elevated levels of aldosterone alone can be implicated in premenstrual edema, and present data suggest that the effect of PRL on fluid retention is minimal. Several lines of evidence suggest the possibility that elevated VP levels or enhanced VP sensitivity may be involved in cases of premenstrual edema. The role of angiotensin in this disorder requires further study. The recent investigations supporting a natriuretic role of DA and demonstrating low DA excretion in other conditions associated with sodium and water retention have stimulated renewed interest in the role of renal catecholamines on body fluid balance. At present, there are little data concerning the status of renal catecholaminergic mechanisms in PMS.

#### **New dimensions in the understanding of PMS: Neurointermediate lobe peptides**

**$\alpha$ -Melanocyte-stimulating hormone ( $\alpha$ -MSH).** In recent years substantial interest has been directed to the potential role of  $\alpha$ -MSH in humans.<sup>255</sup> This peptide is believed to be produced in the pars intermedia and anterior pituitary by cleavage of the first 13 amino acids from the adrenocorticotrophic hormone molecule.<sup>256</sup> Release from the pituitary is controlled, in part, by the balance of hypothalamic releasing and release-inhibiting factors, neither of which has been characterized to date.<sup>255</sup> In rats, a significant diurnal variation in  $\alpha$ -MSH concentration has been demonstrated within the hypothalamus and median eminence.<sup>257</sup>

Sex steroids appear to play a significant role in the regulation of  $\alpha$ -MSH synthesis and release in rats.<sup>255, 258-261</sup> Evidence from studies during the estrous cycle in these animals suggests that estrogen promotes synthesis but inhibits release of  $\alpha$ -MSH while the major effect of progesterone is to enhance release. Consistent with these findings is the observation in human pregnancy that plasma  $\alpha$ -MSH levels are increased during the third trimester in 50% of subjects.<sup>262</sup> Release of  $\alpha$ -MSH



prior to the menses has been suggested as an explanation for subjective reports of increased skin pigmentation at this time.<sup>263</sup>

Gonadal hormones may influence  $\alpha$ -MSH release through induced changes in DA turnover within the tuberoinfundibular dopaminergic system. Animal studies have demonstrated certain striking similarities between the control of PRL and  $\alpha$ -MSH secretion.<sup>264, 265</sup> DA and its agonists cause a reduction in plasma  $\alpha$ -MSH while DA receptor blockade has the opposite effect. The fact that dopaminergic agonists can suppress plasma  $\alpha$ -MSH levels despite isolation of the pituitary from hypothalamic control<sup>265</sup> and the demonstration that tuberoinfundibular dopaminergic neurons innervate the intermediate lobe<sup>266</sup> are consistent with the view that DA exerts its effect on  $\alpha$ -MSH release by a direct pituitary action. Through short-loop feedback  $\alpha$ -MSH may affect release of DA in a manner similar to that previously reported for PRL.<sup>267</sup>

Until recently little was known about the role of  $\alpha$ -MSH in humans. While MSH in lower vertebrates is important for adaptive color changes it seems to have lost this function in man. Through evolution, however,  $\alpha$ -MSH appears to have retained some influence over adaptive behavior in humans through its ability to increase awareness of environmental stimuli and to enhance arousal and attention.<sup>268</sup>  $\alpha$ -MSH has an important sebotrophic role in mammals<sup>269</sup> which has not yet been confirmed in man. The recent discovery that  $\alpha$ -MSH can cause increases in the levels of growth hormone (GH)<sup>270</sup> and gonadotropins (luteinizing hormone [LH] and follicle-stimulating hormone)<sup>271</sup> in humans and observations suggesting a possible interaction between  $\alpha$ -MSH and VP release in animals<sup>255, 272, 273</sup> lend credence to the postulate that this intermediate lobe peptide may subserve important neuroendocrine functions.

While there is presently no direct evidence to suggest a role for  $\alpha$ -MSH in the pathogenesis of the PMS, this peptide is significantly influenced by gonadal steroids and in concert with the other intermediate lobe peptide  $\beta$ -endorphin, it may modulate mood, behavior, and the integrated function of both anterior and posterior pituitary.

**Endorphins.** The term endorphin has been used to describe a class of endogenous opiate peptides (EOP). The two EOPs which have been most extensively studied include the 31 amino acid  $\beta$ -endorphin ( $\beta$ -LPH/61-91) and the pentapeptide enkephalins although many other fragments with endogenous opiate activity have now been isolated.<sup>274, 275</sup> These EOPs are widely distributed throughout the body, not only within the brain-pituitary system but also at other sites, including

the adrenal, pancreas, and gastrointestinal tract.<sup>276, 277</sup>

There is increasing evidence to suggest that EOPs may function as neurotransmitters or neuromodulators to regulate, among other things, the release of hormones from the pituitary. In animals, endorphins have been reported to produce dramatic increases in levels of GH and PRL while decreasing the concentration of circulating LH.<sup>278</sup> The opiate antagonist naloxone specifically blocks these responses.<sup>278</sup> In normal male and female subjects intravenous  $\beta$ -endorphin causes an increase in the concentration of PRL and a decrease in the levels of GH and LH.<sup>279</sup> A chronic inhibitory effect of endogenous opiates on gonadotropin secretion in humans may be deduced from the finding that the opiate receptor antagonist naloxone causes an increase in LH concentration in female subjects<sup>280</sup> which is associated with an increase in the frequency and amplitude of pulsatile LH release.<sup>281</sup> LH suppression is maximal during the luteal phase, suggesting that endorphin activity may be highest at this time. Indeed, the rise in LH levels following naloxone is significantly greater in the midluteal than in the early follicular phase of the cycle.<sup>280</sup>

EOPs may affect the release of VP. Morphine in animals and man has long been known to have an antidiuretic effect. VP release following endorphin administration has been reported in both rats<sup>282</sup> and rabbits.<sup>283</sup> The facts that the intermediate lobe contains the highest levels of  $\beta$ -endorphin in the pituitary<sup>284</sup> and that a depletion of neurointermediate lobe opioid-like peptides occurs in rats during the process of dehydration<sup>285</sup> add further support to the possibility that endorphins may play a physiologic role in the modulation of VP release. In humans, the opiate antagonist naloxone decreases both basal and stimulated VP release, suggesting that endogenous opioids may act as a tonic stimulus for VP release.<sup>286</sup>

The effects of endogenous opiates on glucose metabolism are currently under investigation. For years it has been known that acute administration of morphine to dogs causes hyperglycemia while chronic administration may produce a paradoxical hypoglycemic response<sup>287</sup> associated with hyperinsulinism.<sup>288</sup> Both in vitro studies in the isolated perfused dog pancreas<sup>289</sup> and recent in vivo studies in humans<sup>290</sup> have demonstrated that exogenous  $\beta$ -endorphin will elicit prompt release of glucagon and insulin. In humans this results in hyperglycemia with secondary hyperinsulinism.<sup>290</sup> Perhaps increased endogenous opiate activity resulting in pancreatic  $\beta$ -cell hyperfunction with secondary hyperinsulinism could result in hypoglycemic symptoms upon withdrawal of the opiate stimulus.

Endogenous opiates have been shown to have a pro-

found influence on appetite and thirst in rats. The opiate antagonist naloxone will produce a dramatic dose-dependent inhibition of appetite<sup>291</sup> and thirst,<sup>292</sup> even in animals under conditions of food and water deprivation.<sup>293</sup> In contrast, administration of opiates will cause a satiated rat to resume eating and drinking behavior sometimes until it becomes obtunded.<sup>294</sup>

There is now evidence to suggest that opiates may inhibit the actions of prostaglandin  $E_1$  ( $PGE_1$ ). Inhibition of  $PGE_1$ -stimulated fluid secretion by the small intestine has been suggested as one factor contributing to the constipating effects of opiates<sup>295</sup> while inhibition of  $PGE_1$ -stimulated generation of cyclic adenosine monophosphate has been proposed as a mechanism contributing to opiate-induced analgesia.<sup>296</sup>

Initially, interest in endorphins centered about the possibility that they might be a potent nonaddicting analgesic. While they were indeed potent as an analgesic, animal studies demonstrated that withdrawal effects could be seen after the termination of relatively short-term infusions (70 hours).<sup>297</sup> Efforts to understand the mechanisms for the phenomena of tolerance and withdrawal in narcotic addiction have led to the development of an intriguing model for the action of endorphins within the brain.<sup>298-300</sup> The demonstration that dopaminergic neurons possess opiate receptors has provided an important link between mood and behavior and the neuroendocrine function of the pituitary. In general, the activation of presynaptic opiate receptors on these and other neurons of biogenic amine systems by exogenous or endogenous opiates leads to a decreased cell firing rate. This decreased activity across the synapse in turn produces a disuse hypersensitivity of the postsynaptic nerve terminal such that tolerance (or accommodation to the decreased level of neurotransmission) develops, without a change in the number or affinity of opiate receptors. When the opiate stimulus is withdrawn, resumed neurotransmission and receptor supersensitivity lead to a temporary overactivity of the postsynaptic neuron which results in withdrawal symptoms.<sup>298-300</sup> Decreased neurotransmission of biogenic amines (norepinephrine and dopamine) has been implicated in the pathogenesis of depression<sup>301, 302</sup> while excessive activity has been linked to irritability, aggression, and psychosis.<sup>303-305</sup> The hyperirritability and spontaneous aggressive behavior which are common during acute opiate withdrawal in animals and man vary in intensity, depending on the duration of opiate exposure and rapidity of withdrawal.<sup>303, 304</sup> These fairly specific responses to opiate withdrawal appear to result from dopaminergic hyperfunction because dopaminergic agonists enhance and dopamine receptor blockers reverse this behavior.<sup>302</sup>

In its most severe form such dopaminergic hyperfunction may result in the development of psychosis.<sup>305</sup>

**Dysfunction of the neurointermediate lobe in the PMS—A hypothesis.** We postulate that an aberrant release of, or sensitivity to, the neurointermediate lobe peptides  $\alpha$ -MSH and  $\beta$ -endorphin during the luteal phase may be the central event which triggers a cascade of neuroendocrine changes leading ultimately to the varied manifestations of the PMS. Recent studies on the effects of these intermediate lobe peptides have revealed that they elicit a variety of neuroendocrine responses involving both the adenohypophysis and neurohypophysis. Their unique location within the brain as well as the anterior and intermediate lobes of the pituitary suggests that they may participate in the modulation or integration of mood, behavior, and humoral responses within the brain-pituitary complex. The cyclic changes in  $\alpha$ -MSH secretion in response to gonadal steroids and the preliminary evidence suggesting a relationship between  $\alpha$ -MSH,  $\beta$ -endorphin, and VP constitute a highly attractive area for future exploration of the PMS. Endorphin-induced inhibition of central biogenic amine systems may produce mood changes, increased appetite, and thirst. Mastodynia, fluid retention, constipation, and abdominal bloating in individual cases may result from endorphin-incited increases in the levels of PRL and VP and the inhibitory effect of endorphins on  $PGE_1$  action within the bowel. Withdrawal of the endogenous opiate stimulus affords relief from these complaints but may trigger new symptoms. Increased activity of biogenic amine systems may produce rapid improvement in mood and relief from mastodynia, edema, and bloating. Release from opiate inhibition of  $PGE_1$  action may explain the headaches and loose stool which frequently accompany the onset of menstruation. In exceptional cases an excessive rebound to dopaminergic hyperfunction may explain the development of late premenstrual hyperactivity, irritability, aggressive and hostile behavior, or psychosis. Although the factors determining the degree and duration of endogenous opiate exposure and the rate of withdrawal are unknown, it is likely that gonadal steroids are involved. Differing levels of gonadal steroids from month to month and from person to person may account for the heterogeneous and variable clinical manifestations in this disorder.

### Comment

Distressing premenstrual symptoms afflict a major proportion of the female population. In a significant percentage of these women the cluster of symptoms is severe enough to cause temporary disruption of inter-



personal relationships and on-the-job effectiveness.

The literature on the premenstrual syndrome has been reviewed to evaluate existing theories, to highlight areas requiring clarification, and to extract clues to assist in the formulation of a new hypothesis.

To date no one hypothesis has adequately explained the constellation of symptoms composing the PMS. Estrogen excess or progesterone deficiency, while sometimes present in PMS, appears to be secondary to other neuroendocrine changes such that therapy directed toward manipulating sex steroid levels has met with limited success. Oral contraceptives relieve the dysmenorrhea frequently associated with the PMS but fail to alleviate uniformly the symptoms of the premenstruum.

There is no apparent rationale for vitamin A therapy in PMS and this should be abandoned. The use of vitamin B<sub>6</sub> remains controversial, for the reported beneficial effects of this agent on dopamine and serotonin metabolism are highly inconsistent. Unless a double-blind trial can demonstrate the efficacy of this therapy for PMS it should be considered only as a safe placebo treatment.

Glucose metabolism may be under the cyclic influence of sex steroid hormones but this area requires further investigation. A rebound from increased glucose tolerance in the midluteal phase may account for the craving for sweets or increased appetite so frequently reported in the premenstruum.

Endogenous hormone allergy is an established phenomenon in dermatology. Whether such a phenomenon participates in the PMS remains suspect because of the unreliable nature of skin testing procedures employed in past studies. Clearly the effects of sex steroid

hormones on the human allergic response need further study.\*

The fact that emotional factors may induce neuroendocrine changes has been established in other psychoneuroendocrine disorders such as hypothalamic amenorrhea and pseudocyesis. In the PMS although there is little doubt that core psychological characteristics may modulate an individual's interpretation or expression of premenstrual symptoms, there is little to support the belief that psychological factors alone incite all other symptoms of the PMS.

Many past investigators have suggested that fluid retention caused all the symptoms of PMS. It now appears that fluid retention is not always present in the PMS and a reinterpretation suggests that it is just one manifestation of an underlying neuroendocrine disorder. In the cases where there is undoubtedly excessive cyclic fluid retention no one hormone has been clearly implicated. Angiotensin may alter capillary permeability, cause central release of VP, and induce thirst but whether this contributes to PMS is unknown. Reports that aldosterone levels are normal in PMS argue against this hormone's playing a major role. There is presently little evidence to support the premise that prolactin causes fluid retention in humans. Aberrant renal dopaminergic function may be primary, or secondary to some other factor causing fluid retention. VP remains a strong but untested possibility as the fluid-retaining agent.

Based on the foregoing information concerning the central and pituitary role of the neuropeptides  $\beta$ -endorphin and  $\alpha$ -MSH, a new hypothesis for the pathophysiology of PMS has been proposed.

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## COMMUNICATIONS IN BRIEF

This section is suitable for reporting results of therapeutic trials, descriptions of new procedures or instruments, and case reports which illustrate a principle. Reports should be limited to seven hundred words and two references. Use of an illustration or table requires a proportionate reduction in total words.

### Endometriosis in a patient with Rokitansky-Kuster-Hauser syndrome

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THE HISTOGENESIS of endometriosis has been explained by several theories, the most widely accepted of which is that of tubal reflux menstruation as proposed by Sampson over 50 years ago. This case report documents the occurrence of endometriosis in a patient with Rokitansky-Kuster-Hauser syndrome.

A 23-year-old woman was seen for the evaluation of primary amenorrhea. The patient, the product of an uneventful pregnancy, had been born to a 26-year-old mother. She had a younger sister, age 21, who experienced regular menstrual periods. Physical development at puberty was uneventful. She had no complaints of cyclic abdominal pain.

Physical examination disclosed that the patient was an intelligent young woman who weighed 112 pounds and was 63 inches tall. Development of the breasts and pubic hair was normal, as was the appearance of the external genitalia. No skeletal abnormalities were seen. The vagina ended in a blind pouch, 1 cm in depth.

Findings of laboratory examinations included normal gonadotropins, biphasic basal body temperature chart, a normal 46,XX karyotype, and a normal intravenous pyelogram.

The patient was admitted for a vaginoplasty by the combined abdominal-perineal approach, with the creation of a canal over a silicone rubber mold and the use of the müllerian

remnants and pelvic peritoneum to cover the mold. At the time of operation, a 4 cm endometrioma was removed from the left ovary. No other areas of endometriosis were noted at that time. The right ovary appeared to be normal, and normal tubes were attached to two small (2 cm in diameter) uterine buds.

The patient had an uneventful postoperative course, and she subsequently experienced normal sexual function. The patient was seen again 2 years later with persistent abdominal pain and a pelvic mass which was confirmed by sonography. A second laparotomy was performed and two large endometriomas were removed. Endometriosis was confirmed histologically. The patient's postoperative course was unremarkable.

The etiology of endometriosis is uncertain. Some investigators favor the theory of metaplasia of the coelomic epithelium. The most widely accepted theory, that of menstrual reflux as proposed by Sampson,<sup>1</sup> is supported by the observation of tubal regurgitation during menses, the ability of transplanted endometrium to grow experimentally, and the frequent occurrence of endometriosis in patients with obstruction of menstrual flow by congenital or acquired genital abnormalities.<sup>2</sup>

The occurrence of endometriosis in patients with the Rokitansky-Kuster-Hauser syndrome (characterized by congenital absence of the vagina, bilateral rudimentary uterine horns, normal tubes and ovaries, normal female secondary sexual characteristics, and normal cytogenic and hormonal findings) is unique in that these patients do not have functioning endometrial tissue. Therefore, it is unlikely that tubal regurgitation is the only cause of endometriosis.

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## Culture-proved Fitz-Hugh–Curtis syndrome

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AN UNCOMMON SEQUELA of genitourinary gonococcal infections which is seen almost exclusively in women is the Fitz-Hugh–Curtis syndrome. Although the syndrome was first described in 1919, and many cases have been reported since, the proposed etiologic agent, *Neisseria gonorrhoeae*, has never been isolated in cultures from the peritoneal cavity.<sup>1</sup> This lack of culture-proved cases has led some investigators<sup>1</sup> to speculate that other organisms may be partly or wholly responsible for this syndrome, and others<sup>2</sup> to doubt that the gonococcus directly traverses the peritoneal cavity en route to the liver. In this article, we present the first report of a patient with the Fitz-Hugh–Curtis syndrome in whom *N. gonorrhoeae* was isolated from both endocervical and peritoneal cultures, and we also emphasize that this syndrome may occur after abdominal trauma and mimic an acute surgical emergency.

A 24-year-old woman was admitted to St. Elizabeth's Hospital with a 1-day history of severe pain in the right upper quadrant of the abdomen, associated with pleuritic pain in the right lower side of the chest and in the right shoulder, subsequent to abdominal trauma that was secondary to physical abuse. On admission, the patient was in acute distress because of abdominal pain. The physical examination disclosed a blood pressure of 90/70 mm Hg without postural changes, a pulse rate of 120, and a temperature of 101.2° F orally. The chest and cardiovascular systems exhibited no abnormalities. Examination of the abdomen revealed a lack of bowel sounds, a diffusely tender abdomen with exquisite tenderness in the right upper quadrant associated with localized voluntary guarding, and rebound tenderness in this area. Findings on examination of the pelvis included a mildly tender cervix with a white discharge at the cervical os.

Initial laboratory data showed a hematocrit of 29% and white blood cell count of 6,000. Electrolytes, blood urea nitrogen, and amylase were normal. Liver function tests exhibited mild abnormalities with an alkaline phosphatase of 238 IU per liter, a serum glutamic pyruvic transaminase of 34 IU per liter, and a serum glutamic oxaloacetic transaminase of 78 IU per liter. Roentgenograms of the chest, ribs, and abdomen were unremarkable.

Because of the clinical presentation of an acute condition in the abdomen, associated with a recent history of abdominal trauma and a low hematocrit, an abdominal tap was per-

formed. No blood or peritoneal fluid was obtained initially. However, lavage with 200 ml of nonbacteriostatic sterile saline solution yielded the return of a yellow, cloudy fluid. The cell count of the fluid revealed no red cells and 1,230 white cells per milliliter with a differential cell count of 100% polymorphonuclear leukocytes. Cervical discharge and peritoneal lavage were cultured on chocolate agar, sheep blood agar, EMB agar, and in thioglycollate broth. With evidence of peritonitis, therapy with clindamycin and gentamicin was begun. On the second hospital day, reports on the cultures indicated that both peritoneal and endocervical cultures were growing *N. gonorrhoeae*, confirmed by cysteine tryptic agar sugars. Cultures of urine and blood were negative. No change in the patient's clinical status had occurred since the time of admission. Intravenous ampicillin was added to the patient's regimen. The patient showed remarkable improvement and was free of symptoms after 6 days of ampicillin therapy.

Clearly, this patient was suffering from the Fitz-Hugh–Curtis syndrome. The symptom complex, consisting of the acute onset of severe pleuritic pain in the right upper quadrant of the abdomen, with radiation to the right shoulder and around to the back, in a patient with genitourinary gonorrhea, was synonymous with the classic clinical descriptions of the disease. Of interest is the fact that this case occurred after abdominal trauma and mimicked an acute surgical condition in the abdomen.

Since the original description of the Fitz-Hugh–Curtis syndrome, investigators have pondered the route of spread of the gonococcus from the genitourinary system to Glisson's capsule. Initially, the mechanism of spread was thought to be a direct one from a genitourinary focus through the fallopian tubes and via the peritoneal cavity to the liver. However, despite a multitude of case reports which have documented the existence of the clinical syndrome and its association with genitourinary gonococcal infections, the gonococcus has never been retrieved from peritoneal cultures.<sup>1</sup> In light of this, and the fact that two cases of perihepatitis have been documented in male patients,<sup>2</sup> mechanisms of spread to the liver capsule other than by direct extension from the fallopian tubes have been hypothesized.<sup>2</sup> Similarly, other investigators have presented evidence which suggests that *Chlamydia trachomatis* may be involved in the pathogenesis of many, and perhaps most, cases of this syndrome.<sup>1</sup>

In summary, this case clearly demonstrates that the Fitz-Hugh–Curtis syndrome may be caused solely by an ascending gonococcal infection in female patients, and it substantiates earlier theories that the gonococcus is the etiologic agent in this syndrome and that it reaches the liver capsule by direct spread across the peritoneal cavity. Lavage of the peritoneal cavity with saline solution, as performed in this patient, may increase the yield of positive peritoneal cultures in cases of this syndrome, particularly if the material obtained is cultivated on appropriate media. Obviously, if unnecessary

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surgical procedures are to be avoided, it is essential that physicians be aware of the ability of this syndrome to mimic an acute condition in the abdomen, particularly after trauma.

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Correlation of daily fetal movements and the nonstress test as tools for assessment of fetal welfare

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THE ASSOCIATION of acceleration of the fetal heart rate with fetal movements (the nonstress test) and the presence of daily fetal movements have been shown to be helpful in assessing antenatal fetal welfare.<sup>1, 2</sup> Both tests have their greatest accuracy in predicting a healthy fetus. The limitations are their reduced accuracy in identifying a compromised fetus.

This prospective study was designed to evaluate the relationship of two fetal biophysical variables, the nonstress test (NST) and the daily fetal movement record (DFMR), and to compare the accuracy of combining the two in predicting antenatal fetal welfare and not intrapartum or postpartum complications.

A 1-year prospective study of 237 high-risk prenatal patients was performed. All patients volunteered for the study after a careful explanation of the protocol. The women were provided a form on which to record, three times each day for 30 minutes, daily fetal movements. They were instructed to lie down in a comfortable position for three uninterrupted periods. Movements were recorded in the morning, afternoon, and evening. The results were collected each week, and a new form was provided. Reduced fetal movements were defined as less than 0 to 5 movements per 30 minutes for each of the three periods (a modification of the Sadovsky-Yaffe method).<sup>1</sup> If there were reduced fetal movements, the patients were instructed to notify a member of the perinatal team.

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Table I. Comparison of daily fetal movement record (DFMR) and the nonstress test (NST)

| NST              | DFMR        |            |
|------------------|-------------|------------|
|                  | Adequate    | Reduced    |
| Reactive (224)   | 183 (94.3%) | 41 (95.5%) |
| Nonreactive (13) | 11 (5.7%)*  | 2 (4.5%)*  |
|                  | 194 (81.9%) | 43 (18.1%) |

\*No statistical significance.

A nonstress test was performed\* at each visit. Testing was usually begun at 32 weeks and repeated at weekly intervals unless changes in the status of the mother and the fetus dictated that the pregnancy should be interrupted. To be included in the present analysis, all patients had to have had their last tracing within 1 week of delivery. A reactive NST was defined according to Rochard and associates.<sup>2</sup>

The number of DFMRs varied from as few as 7 days to as many as 35 days. The 237 patients had 441 NSTs. For purposes of analysis, only the last NST and DFMR performed within 7 days of delivery were considered. Since antenatal fetal surveillance techniques do not necessarily predict intrapartum problems, these were not considered in a comparison of the two modalities. There were no antenatal fetal losses.

Overall, fetal movements were adequate in 81.9% of observations, and the NST was reactive in 94.5% of these patients. Fetal movements were adequate in 94.3% of the women with a reactive NST and were adequate in 95.5% with a nonreactive NST. Conversely, fetal movements were reduced in 5.7% of the patients with a reactive NST and were reduced in 4.5% of the patients with a nonreactive NST (Table I). Statistical analysis of these results showed that the presence of daily fetal movements was not very sensitive nor specific for the detection of antenatal fetal jeopardy when the two groups were compared. Statistical analysis indicated that the NST and DFMR were not related ( $\chi^2_1 = 0.01$ ;  $p > 0.90$ ).

The frequency of a nonreactive NST is essentially the same whether fetal movements are adequate or reduced. Statistical significance was examined by means of Fisher's exact probability test. The observations were well below levels of significance. Thus, the accuracy of the DFMR in predicting uteroplacental insufficiency is negligible.

In this prospective study, we have measured two variables in the same patient and noticed remarkable dissimilarities between the two in the predictive accuracy of the DFMR. Thus, it is apparent that the combination of these two modalities offers very little help to the clinician.

\*Brattle Instrument Corporation.

It was hoped that the combination of the two tests would provide more information relevant to antenatal fetal welfare. The DFMR has certain obvious advantages: availability, convenience, simplicity, and inexpensiveness. Perhaps with the development of special instrumentation, i.e., ultrasound, the accuracy of the DFMR can be improved.

Although decreased fetal movement may alert the clinician to further evaluation of the fetal condition, the DFMR should not be used as the sole screening device to determine fetal welfare.

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### Recovery of *Neisseria meningitidis* from the cul-de-sac of a woman with endometritis-salpingitis-peritonitis

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AN UNCOMMON ISOLATE from the female genital tract is *Neisseria meningitidis*.<sup>1, 2</sup> Inclusion of it in the potential bacterial flora of the female genital tract emanates from isolated instances in which bacteria have been identified or recovered from the female genital tract, independent of local or systemic disease.

Unlike the nasopharynx or oropharynx, the female genital tract rarely harbors nonpathogenic *Neisseria* species. The Center for Disease Control accepts the presumptive criteria for *Neisseria gonorrhoeae* (the presence of gram-negative, oxidase-positive diplococci) as being definitive criteria for any isolate obtained from the endocervix. *N. meningitidis* is distinguished from *N. gonorrhoeae* primarily on the basis of its ability to ferment maltose. Since neither carbohydrate fermentation nor immunofluorescence is required for isolates of the female genital tract, except in research laboratories, perception of a possible role for *N. meningitidis* as a pathogen for the female genital tract has been effectively negated. Every gram-negative, oxidase-positive diplococcus recovered from the endocervix is automatically presumed to be *N. gonorrhoeae*.

The use of culdocentesis in conjunction with the ap-

plication of anaerobic biotechnology has demonstrated that, in certain patients with gonococcal endocervicitis and the clinical findings of endometritis-salpingitis, a polymicrobial peritonitis may evolve from which *N. gonorrhoeae* may or may not be isolated.<sup>3-6</sup> Reported in this article is the recovery of *N. meningitidis* from the cul-de-sac of a woman who presumably had gonococcal endocervicitis and clinical evidence of salpingitis-peritonitis.

The patient was a 25-year-old black woman, para 3-0-0-3, whose last menstrual period occurred 1 week prior to the onset of crampy pain low in the abdomen bilaterally. There was no associated nausea, vomiting, diarrhea, or constipation. The patient denied prior episodes of endometritis-salpingitis; however, she acknowledged orogenital sex. Physical examination revealed marked deep organ and rebound tenderness; the bowel sounds were high pitched. The abdomen was slightly distended. Pelvic examination was inadequate because of abdominal guarding, and fullness was identified in the left adnexa. Gram stain of the endocervix revealed the presence of gram-negative intracellular diplococci (GNID). Culdocentesis produced 3 to 4 ml of purulent material. Gram stain of the culdocentesis fluid revealed the rare presence of gram-negative diplococci and rods. Subsequent modified Thayer-Martin cultures grew out morphologically typical colonies which contained gram-negative diplococci that gave a positive oxidase reaction. Because the white blood cell count was 11,700/cu mm, the patient was given doxycycline. The temperature of 101.9° F at the time of admission abated rapidly over the ensuing 10 hours; however, on the second hospital day, there was a secondary febrile spike to 100° F, and by the third hospital day the patient had sustained a low-grade temperature between 99.8° and 100° F. At the time of the second febrile spike, the white blood cell count was 16,200/cu mm, and the doxycycline was replaced by ampicillin and gentamicin, with resolution of the febrile morbidity in the ensuing 10 hours. At the time of the patient's discharge, the white blood cell count was 7,400/cu mm.

The posterior vaginal pool was dried with "drumsticks" prior to washing with povidone-iodine solution and a placement of povidone-iodine at the site of proposed penetration. Culdocentesis was performed with an 18-gauge needle. The specimen was subdivided into two equal aliquots which were injected into Anaport\* vials. Care was taken to exclude the presence of any air in the needle. The specimens were processed in an anaerobic glove box by meticulous anaerobic culture methodology.<sup>5, 7</sup> Five bacterial isolates were obtained: alpha nonhemolytic streptococcus (not Group D), *Haemophilus vaginalis* (*Gardnerella vaginalis*), *Peptococcus asaccharolyticus*, *Bacteroides* species, and a strain of *Neisseria* characterized by fermentation of glucose and maltose and nonfermentation of fructose, sucrose, and starch. Beta hemolysis occurred on 5% horse blood agar after 48 hours of incubation. The isolate produced some colonies on Dubos oleic acid agar medium. The strain of *Peptococcus asaccharolyticus* had a minimum inhibitor concentration value (12.5 µg/ml) which exceeded the therapeutic range of doxycycline (4 µg/ml).

Prior to 1974, when the Center for Disease Control

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implemented the policy of accepting the presumptive criteria for the definitive criteria, *N. meningitidis* was isolated on rare occasions as a constituent of the vaginal flora. Reliance on presumptive criteria for the identification of *N. gonorrhoeae* in anogenital specimens taken from homosexual men has been shown to be inappropriate.<sup>9</sup> When presumptive isolates were subjected to carbohydrate fermentation testing, 16.3% and 31% of the isolates presumed to be *N. gonorrhoeae* from two divergent populations were found to be *N. meningitidis*. The unanswered epidemiologic question is whether *N. meningitidis* is an exogenous pathogen that can effectively produce infection in the female genital tract. The organism has been isolated from the male urogenital tract.<sup>8</sup> Gregory and Abramson<sup>2</sup> suggested that the meningococcus could be an etiologic agent for rare cases of vaginitis. They recovered *N. meningitidis* from a 5-year-old girl who had a clinically significant vulvovaginitis. In the case reported, *N. meningitidis* was recovered from the cul-de-sac of a patient who presumably had gonococcal endocervicitis. It is probable that the organism isolated from the endocervix and the organism recovered from the cul-de-sac were one in the same. Irrespective of this postulate, *N. meningitidis* was recovered from the cul-de-sac of a patient with endometritis-salpingitis-peritonitis (ESP). In this situation, it functioned as a constituent of a polymicrobial peritonitis.

In 1976, Monif and associates<sup>10</sup> demonstrated, on the basis of culdocentesis, that the Group A beta-hemolytic streptococci were capable of producing a disease pattern indistinguishable from gonococcal endometritis-salpingitis-peritonitis. A second case of ESP due to the Group A beta-hemolytic streptococci was reported by Eschenbach and associates.<sup>4</sup> The isolation of the Group A beta-hemolytic streptococci, and now *N. meningitidis*, from the cul-de-sac is consistent with the current concept that exogenous bacteria may gain access to the female genital tract and produce local disease. This access may be the consequence of changing sexual practices.

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## Paravestibular tumor of the female genital tract

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PARAVESTIBULAR TUMORS of the female genital tract are very rare, benign lesions.<sup>1-3</sup> It is often difficult to differentiate these lesions histologically from hemangiopericytomas, which frequently are malignant, and from fibroepithelial polyps. Described here is a case of paravestibular tumor of the vagina.

A 22-year-old, single, white woman, gravida 0, with regular menses was seen by her local gynecologist because of a polypoid mass protruding through the introitus. The mass measured 3 by 2 by 2 cm, its base was located 1 cm behind the hymenal ring, and it seemed to arise from the rectovaginal septum. The top of the mass was excised, and pathologic examination of it indicated a polyp with sarcomatous changes. The patient was then referred to the Gynecologic Oncology Service of the North Carolina Memorial Hospital for further evaluation. The patient did not have a history of exposure to diethylstilbestrol. Examination revealed that the introitus and hymenal ring were without lesions, but on the posterior vaginal wall, 1 cm inside the hymenal ring, a nodule, 1.5 by 2 cm, was palpated. There were no other abnormalities. All work-up

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**Fig. 1.** Microscopic view of tumor. ( $\times 200$ .)

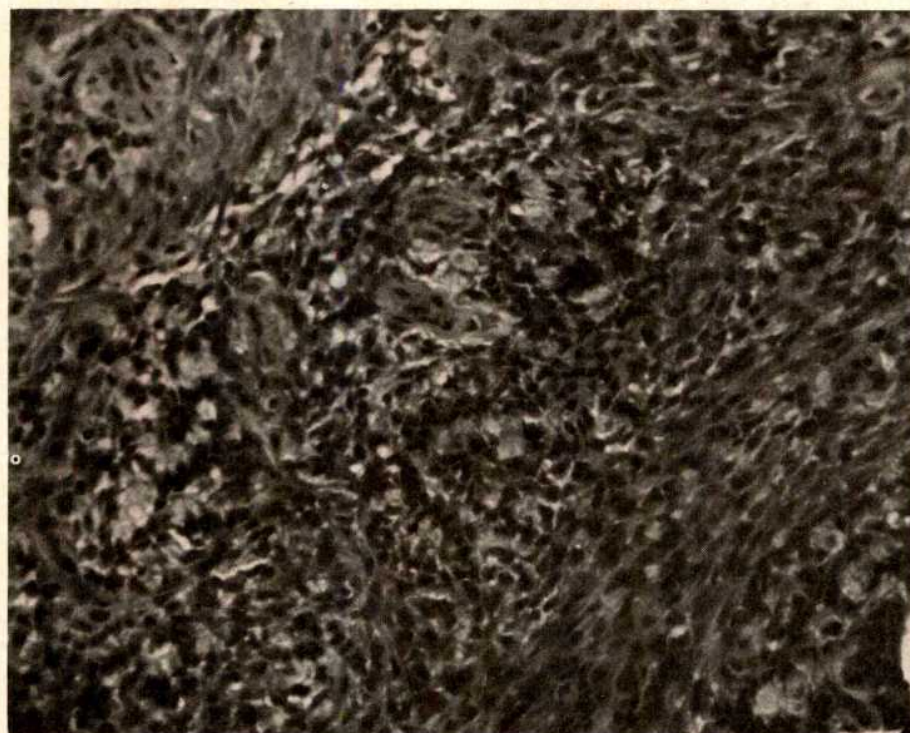


**Fig. 2.** Stroma of tumor. ( $\times 200$ .)



findings were normal (hemogram, liver function tests, intravenous pyelogram, barium enema, bone scan, chest x-ray film, cystoscopy, and sigmoidoscopy). Colposcopic examination produced normal findings. A curettage, cervical biopsies, and excisional biopsy of the portion of lesion remaining in the rectovaginal septum were performed.

Histologic sections of the tumor revealed a circumscribed tumor composed of cells with clear cytoplasm and bland nuclei arranged in acini. The tumor also contained foci of squamous metaplasia and spindle-cell and myxomatous stromal components (Figs. 1 and 2).

Histologic evaluation of the curettage specimen and the cervical biopsy specimens indicated no malignancy. The preliminary report on the tumor by the pathologist at our institution was malignant mixed müllerian tumor.

The slides of the patient's original biopsy specimen were also reviewed by five leading pathologists. Their diagnoses were: a benign polypoid paravestibular tumor (three pathologists), mixed tumor with questionable low-grade malignancy in the stromal component (one pathologist), and heman-giopericytoma (one pathologist). The final pathologic diagnosis made at North Carolina Memorial Hospital was benign paravestibular tumor.

The patient was examined every 3 months without any problems until 42 months after the original diagnosis, at which time a polypoid mass in the same location as the original mass was found on pelvic examination. It measured 2 by 3 by 3 cm. She was hospitalized and the lesion was excised locally. This tumor had the same histologic appearance as the original tumor. The patient has been observed for 24 months since excision of the second lesion and has been without problems.

Paravestibular tumors are characteristically benign and usually arise in the vicinity of the introitus. These tumors are extremely rare and usually occur in young women, although Bantine and associates<sup>3</sup> recently reported two cases in sisters, 39 and 53 years old. Wide local excision of the tumor is the treatment of choice. Since there may be local recurrence, all patients should be followed periodically.

Paravestibular tumors may be easily misdiagnosed, as evidenced in this study by the different diagnoses made by five leading pathologists. It is important, therefore, that these tumors be differentiated from heman-giopericytomas, sarcomas, or other tumors which may be malignant. Misdiagnosis of a paravestibular tumor may result in more radical treatment than is necessary.

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## Prenatal diagnosis of diastrophic dwarfism

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DIASTROPHIC DWARFISM is an autosomal recessive type of short-limb dwarfism associated with characteristic deformities of the hands, feet, and ears, progressive scoliosis, and abnormalities of the joints. Children with this condition have normal intelligence.<sup>1</sup> Although a number of cases have been described postnatally, we do not know of any case diagnosed in early pregnancy. We now report the prenatal diagnosis of a case of diastrophic dwarfism which was made by ultrasonography at 20 weeks' gestation and was confirmed by examination of the aborted fetus.

A 24-year-old Japanese woman, gravida 3, para 2, married to a 25-year-old American, came for genetic counseling at 20 weeks' gestation because of a previous child with diastrophic dwarfism. The findings of diastrophic dwarfism in that child were typical: severe shortness of stature and micromelia, normal head circumference, bilateral deformities of the ear cartilages (cauliflower ears), cleft palate, bilateral subluxation of the thumbs (which gave the appearance of hitchhiker thumbs), and bilateral clubbed feet. Radiologic findings included a normal skull, hypoplasia of the odontoid process and of the midcervical spine, severe shortening of the long bones with widening of the metaphyses, dislocation of both radial heads, and hypoplastic first metacarpals.

After the parents had been advised that there was a 25% risk of having another child with diastrophic dwarfism, they decided to proceed with an ultrasound examination in an attempt at prenatal diagnosis.

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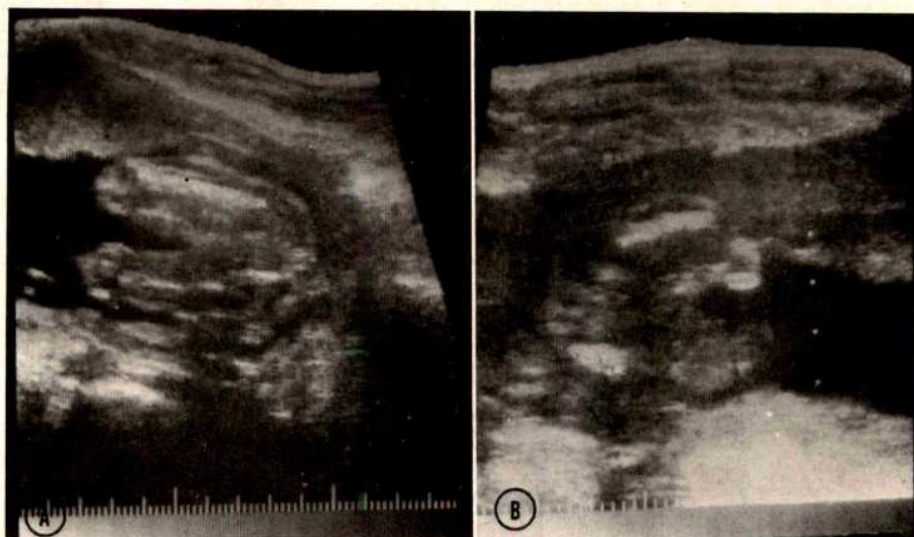


**Fig. 1.** Ultrasound scan through upper legs of a normal fetus (A) and the fetus with diastrophic dysplasia (B).



**Fig. 2.** Full view of the abortus showing the shortness of the extremities and the hitchhiker thumbs.

The ultrasound examination (Fig. 1) was performed with two real-time machines with linear-array transmitters (ADR, Tempe, Arizona, and Narco Air Shield, Harboro, Pennsylvania) and a B-mode static scanner (Picker, Northford, Connecticut). Many scans were obtained of both fetal femurs and humeri. Even before measurements were made, it was apparent on ultrasound that there was rhizomelic and acromelic shortening of all limbs. The maximum femur length was 2.2 cm, compared with a mean of  $3.7 \pm 0.2$  (SD) at 16 to 20 weeks' gestation. An attempt was made with real-time scanning to demonstrate abducted thumbs, but although such a finding was suspected in one scan, this could not be documented by photographs.

After the parents had been informed that the limb length was more than four standard deviations below the mean for gestation, and that there was strong suspicion of an affected fetus, they decided to terminate the pregnancy because they could not deal with the psychological impact of another affected child. Termination of pregnancy was accomplished by intra-amniotic instillation of 20% sodium chloride solution. Complete expulsion of all products of conception occurred 16 hours after instillation.

Examination of the abortus revealed a female fetus with disproportionately short extremities and typical hitchhiker thumbs bilaterally (Fig. 2). The body weight was 332 grams (twenty-fifth percentile for gestational age), the crown-to-heel length was 21.5 cm (mean, 26.08 cm for gestational age), and the head circumference was 16.5 cm. The shoulder-to-wrist length was 4 cm each side, and the hip-to-ankle length was 5 cm each side. Measurements of the bone length of the extremities were as follows: humerus, 2.5 cm; radius, 1.8 cm; ulna, 1.2 cm; femur, 2.5 cm; tibia, 2.3 cm; and fibula, 1.7 cm. No other macroscopic abnormalities were found except those described.

The prenatal diagnosis of skeletal dysplasias by ultrasonography is a rapidly growing field.<sup>2</sup> In the past, the prenatal diagnosis of such disorders was difficult to make because of insufficient experience in ultrasound



techniques, and the lack of normal values for limb measurements in fetuses. Ultrasound diagnosis was enhanced by autopsy-obtained values for bone length of the extremities in fetuses between 16 and 20 weeks' gestation.

Diastrophic dysplasia in this fetus was strongly suspected because of the shortness of the extremities found by ultrasound, and the diagnosis was confirmed by postmortem measurements. Both the shoulder-to-wrist length (4 cm) and the hip-to-ankle length (5 cm) of the fetus were significantly lower statistically ( $p < 0.001$ ) than the values of  $8.2 \pm 0.5$  and  $7.4 \pm 0.4$ , respectively, obtained by Hobbins and Mahoney<sup>2</sup> from external measurements made with a plastic rule on abortuses of 16 to 22 weeks' gestation. The femurs were also 4.3 standard deviations below the mean for gestation when compared with in vivo ultrasound val-

ues obtained from normal fetuses.<sup>3</sup> However, confirmation of the diagnosis in this case was made by the bilateral subluxation of the thumbs found (hitchhiker thumbs), which is so distinctive in cases of diastrophic dwarfism as to be almost pathognomonic of the condition.<sup>1</sup>

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#### **Diagnostic Ultrasound Course for Sonologists and Sonographers**

The Department of Diagnostic Ultrasound at the Health Sciences Centre, Winnipeg, Manitoba, Canada, will offer a special "Advanced Educational Seminar," January 26-31, 1981.

For further information please contact: Ms. Maureen Kotesky, Program Co-ordinator, Section of Diagnostic Ultrasound, 700 William Ave., Winnipeg, Manitoba, Canada R3E 0Z3.

#### **Postgraduate Workshops in Real-time Obstetrical Ultrasonography**

Postgraduate Workshops in Real-time Obstetrical Ultrasonography will be presented by Baylor College of Medicine, Departments of Obstetrics and Gynecology and Radiology, at Jefferson Davis Hospital, 1801 Allen Parkway, Houston, Texas 77019. Dates are Thursday and Friday of each week from January 8-9 through December 17-18, 1981.

For information contact: The Office of Continuing Education, Baylor College of Medicine, Texas Medical Center, Houston, Texas 77030.

#### **Seventh Annual Vail Ob/Gyn Conference**

Beth Israel Hospital of Denver, Colorado, is sponsoring its Seventh Annual Vail Ob/Gyn Conference, February 14 to 21, 1981, at The Mark Resort, Vail, Colorado.

For information write: Beth Israel Conference Program, P. O. Box 11366, Denver, Colorado 80211.

## CORRESPONDENCE

### Recanalization and fistulization of the fallopian tubes are thought to be the causes of pregnancies following female sterilization

To the Editors:

In their recent article, "An epidemiologic study of risk factors associated with pregnancy following female sterilization," Chi and associates<sup>1</sup> found that patients sterilized by electrocoagulation had a significantly lower pregnancy rate than those sterilized with mechanical devices. However, there was a highly significant statistical increase of ectopic pregnancy when electrocoagulation was used. These authors stated that because of the life-threatening aspect of ectopic pregnancy, the possibility of burns, and the difficulty in achieving reversal of sterilization after electrocoagulation, mechanical occlusive devices are most desirable.

With this I must take exception. A recent paper of mine,<sup>2</sup> entitled "High rate of ectopic pregnancy following laparoscopic tubal coagulation failures," confirms the concerns of Chi's group about the problem with ectopic pregnancies after failure of electrocoagulation. However, the purpose of my paper was not to condemn the electrocoagulation type of tubal closure but to change the operator's technique from what was originally taught. Palmer,<sup>3</sup> in 1962, described the classic technique of laparoscopic tubal electrocoagulation of the isthmic portion of the oviducts. Coagulation of the inner one third of the tube has generally been done and this is where the problem lies with future ectopic pregnancies. I am now collecting a series of pathology slides from tubal and cornual areas of hysterectomy specimens which had previous electrocoagulation sterilization. If the tubal damage involves the uterine cornu or the proximal 10 mm of the isthmic portion of the tube, activation of the tubal epithelium occurs with invasion of this epithelium into the tubal muscularis and/or cornual myometrium. These tracts often break through the serosa, causing uterine or tubal peritoneal fistulas. This is the mechanism by which the sperm obtain access to the peritoneal cavity, allowing the potential for an ectopic pregnancy.

In 1930, Sampson<sup>4</sup> originally described the pathologic process of postinjury tubal epithelial activation after studying a uterine cornu in which a previous salpingectomy had been performed. He coined the term "endosalpingiosis." In 1934, Rubovitz and Kobah<sup>5</sup> again described endosalpingiosis that occurred after Madlener procedures if the inner one third of the tube was crushed and tied. In 1955, Rotten<sup>6</sup> found proliferation of the endosalpinx and fistulas following his

cornual resection operation. The exact same pathologic picture is being seen in my series when the isthmic portion of the oviduct is damaged close to the cornu. This activation of the tubal epithelium (endosalpingiosis) is not seen if the oviduct is cauterized at the distal isthmic or middle portion.

Therefore, if the laparoscopists avoid coagulating the proximal isthmic portion of the tube, tubal and/or uterine peritoneal fistulas caused by endosalpingiosis can be avoided, thus reducing the potential risk of ectopic pregnancy. The electrocoagulation type of tubal ligation would then be considered the procedure of choice over mechanical devices because of its significantly decreased failure rate.

Chi and colleagues also mentioned the problem of unintentional burns with electrocoagulation and the difficulty in achieving sterilization reversals. My rebuttal to this is that with the newer low-voltage and bipolar systems used by experienced laparoscopists, accidental burns can and should be avoided. Sterilization reversals after electrocoagulation would not be a problem if the midoviduct is cauterized in only one specific segment.

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### Reply to Dr. McCausland

To the Editors:

Dr. McCausland stated that coagulation of the inner third of the tube is responsible for the high rate of ectopic pregnancy following laparoscopic tubal coagu-



lation. He also asserted that if laparoscopists cauterize the distal isthmus, or midportion, of the tube, the potential for ectopic pregnancies would be reduced. This theory might be correct but needs to be examined with empirical data. Unfortunately, the female sterilization data of the International Fertility Research Program do not provide detailed information on the site of the tube to which the occlusion technique was applied. It should be noted that ectopic pregnancy can occur after sterilization performed by any technique.<sup>1</sup> For the 15 poststerilization ectopic pregnancies reported to the IFRP, eight occurred after laparoscopic electrocoagulation; three, after a Pomeroy procedure via minilaparotomy; two, after culdoscopic application of the tantalum clip; one, after laparoscopic application of the prototype spring-loaded clip; and one, after application of the tubal ring via minilaparotomy. There is a high possibility that in some of these cases the midportion of the tube was involved, particularly in the minilaparotomy cases where the tube is frequently elevated to or through the incision, so that the ampulla can be occluded.

Most previous studies presented the proportion of poststerilization ectopic pregnancies by tubal occlusion technique. While this information is clinically pertinent, it does not convey the *true* risk of a technique leading to an ectopic pregnancy. A technique that is more effective in preventing an intrauterine pregnancy would automatically, but unfairly, be associated with a higher *relative* risk of ectopic pregnancy, although the *true* risk of having an ectopic pregnancy may be similar to that associated with other techniques. In theory, the life-table rates correct this bias, but because of the rarity of poststerilization ectopic pregnancy, it is extremely difficult to conduct a multicenter study with long-term follow-up and with the assurance that all poststerilization ectopic pregnancies are reported.

The question is whether electrocoagulation by means of low-voltage and bipolar systems is a procedure of choice over the mechanical devices. First, the efficacy of these systems in preventing poststerilization pregnancies needs to be evaluated. There is some suspicion that since electrocoagulation with the newer systems is less destructive to the tube than that with the unipolar systems, the failure rate may be higher and similar to that following the application of the mechanical devices. Dr. McCausland assumes that, with these newer systems, the problem of unintentional burn could be avoided if an operator is experienced and does not try to divide the tubes. However, according to the 1975 AAGL survey, some operators using the bipolar systems did divide the tubes and hemorrhage did occur.<sup>2</sup> One bowel burn was also reported.<sup>3</sup>

As for the reversibility of the procedure when the low-voltage, bipolar systems are used, again empirical data are needed. While these systems are less destructive than the unipolar systems, they are still more destructive than mechanical devices.<sup>4</sup> The 1976 AAGL survey reported only 78 patients who requested rever-

sal after bipolar coagulation; in nine cases, reversal was performed. The success rate was not reported.<sup>5</sup>

Finally, our data were primarily from female sterilization procedures performed in developing countries where electricity is not always available and is sometimes unpredictable with voltage fluctuations. In these settings, the mechanical devices are more desirable than the electrocoagulation technique, whether the system is unipolar or bipolar.

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Partial support for this work was provided by the International Fertility Research Program with funds from the Agency for International Development.

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#### Abortion-sterilization by abdominal hysterectomy

To the Editors:

It strikes me as odd and disturbing, in the face of rising criticism regarding unnecessary hysterectomies, that Stumpf, Ballard, and Lowensohn, in their article, entitled "Abdominal hysterectomy for abortion-sterilization" (*Am. J. Obstet. Gynecol.* 136:714, 1980), would propose that we do abortion-sterilization by hysterectomy instead of the tried-and-true, simple, inexpensive, outpatient suction aspiration and laparoscopy. The so-called "indications" for this procedure, including such "social problems" as psychiatric disorders and previous failed sterilization, are new to me as justifications for this major form of surgery. By their own admission, at least 70% of the cases involve uterine specimens with no abnormality.

The fact that the authors had a low morbidity rate in no way justifies this massive, expensive surgical "overkill." It is hard not to conclude that the authors were looking for a way to provide sterilization to Catholic/Spanish patients in a religiously inoffensive way as well as to provide surgical experience for their residents.

The arguments used to justify these procedures are among the most specious I have ever heard. Beyond the highly doubtful "social problems," the entire case

seems to rest on the so-called "poststerilization syndrome," suggesting that women who undergo tubal sterilization have an abnormally high incidence of dysmenorrhea and menorrhagia, which are, in some vague, undefined way, caused by the operation and which frequently require subsequent hysterectomy. The confusion surrounding this issue is emphasized by the wide range of incidences cited by the authors. It should be obvious to all that this is a syndrome that exists largely in the eye of the beholder. In my own practice, I have performed over 400 sterilizations in the past 5 years and no more than four patients have subsequently required hysterectomy.

The problem lies, I believe, in three general areas: (1) postpill anovulatory dysfunctional bleeding (DUB), a generally self-limited problem, rarely requiring hysterectomy; (2) inappropriate selection of candidates for sterilization, resulting in psychogenic DUB; (3) tubal fulguration frequently resulting in damage to ovarian blood supply and subsequent DUB. Hopefully, the latter can be eliminated by switching to nonelectrical, less destructive sterilization techniques.

In conclusion, I am disappointed that a university department of obstetrics and gynecology has sanctioned a paper based on such intellectual dishonesty. Having failed to justify the enormous additional cost of these hysterectomies by any scientifically acceptable medical criteria, the authors should at least be honest enough to argue the true motivating factors behind this paper, i.e., whether there is a need to make hysterectomy for sterilization available electively to those with religious objection to tubal ligation and whether obstetrics and gynecology residents need more surgical experience. I doubt if the public is willing to cough up the extra money for either of these causes.

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#### To the Editors:

The article by Stumpf, Ballard, and Lowensohn was so startling as to content and comment that I could not let it pass without reply. I note that the authors stated that they were not necessarily advocates of the procedure but simply reporting its safety "when it was selected as the method of choice." I begin my reaction to this article by asking Dr. Stumpf (and associates) just how this became a method of choice if the safety was not somehow assured.

At the time of the study period, the rate of hysterectomy was almost two per week over a 6+-year period of time. I was not quite sure what was meant by "Spanish surname" as a designated group as I have never heard any ethnic group described by name. Almost two thirds were under the age of 35. In my hospitals with private

services with mostly North American surnames, a hysterectomy in a patient less than 35 years old is indeed infrequent or even rare and most practitioners use a second opinion in order to clarify the indications because of the youthfulness. In the few very young patients in this study, the indication was clarified by adding various other stated reasons for the procedure. Thus, mental retardation (a diagnosis unclear to the experts and no contraindication to childbirth), cerebral palsy (again, no contraindication); various cardiac diseases (where it seems logically that major surgery would be avoided at all but the highest of indications), and finally psychosis and alcoholism (as nebulous and subjective a diagnosis as can be made and maybe a contraindication to a surgical procedure) were listed as indications. The authors even stated that a certain number had "psychiatric indications for the operation." I must ask Dr. Stumpf to detail what operation would ever be psychiatrically indicated (with the possible exception of prefrontal lobotomy up to the nineteenth century).

"ALL" (their caps) were screened and counseled by staff members and the article stated that detailed informed consent was required prior to surgery. What is interesting is that at this very time there is an appeal being prepared for hearing in a federal court concerning the Los Angeles County Hospital over this same period of time and regarding the issue of informed consent. It would help greatly if the authors explained just what was meant by their "informed consent." Is there such a thing between different cultural groups, people with different surnames and different ethnic backgrounds, and in the mentally ill or psychotic? As Dr. Harold Schulman wrote in an AMERICAN JOURNAL article some years ago, what would any woman say to accepting a procedure that is associated with a 20 times death rate over another procedure that accomplishes the same end? The authors did mention several articles that reported favorably on the subject but glaringly omitted any that were unfavorable.

One had to be impressed with the performance of 500 such operations with such an excellent morbidity rate and an average stay of 5 days. What is a concern is that this may not be so unusual, albeit proud. Hysterectomy for indicated abnormality must always account for in the complication rate the problem of whatever that abnormality had brought. It is no wonder that in otherwise healthy women such good results were obtained.

As to the stated advantages, a 13% failure rate for tubal ligation is manyfold more than the generally accepted one. This has never been a reason for its disuse. Finding future needs and indications for further abdominal surgery have also long been discounted whenever elective procedures are discussed. The appendix remains as the only human redundant organ.

However, the key to my critique is based not on debatable although for me incontrovertible points but on



the whole of the issue. I remain disturbed that we would select a group of women and subject them to a known risk of a major surgical procedure when we all accept that the desired results can be obtained by other means.

In addition, I am concerned by this approach to the subject and that the authors did not see fit to point out the other side of the coin since they were not "advocators of the procedure." The uterus is not, like the appendix, a redundant organ. The negative sequela(e) of such a procedure can be and often is enormous. I refer to my own treatment of the subject in the *GRAY JOURNAL* in 1978 (131:599, 1978).

The authors concluded that "... the choice ... should be considered ... carefully ..." by patient and physician. I regret that the information presented by Dr. Stumpf, Ballard, and Lowensohn did nothing to indicate that such care was taken by them. In the era of medical scrutiny that we are living in, it would be wise for all of us to walk a more secure path.

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#### Reply to Drs. Priver and Sloan

*To the Editors:*

The authors are aware that hysterectomy abortion is a controversial procedure and some physicians may feel it is not warranted; even the subject of hysterectomy for sterilization in nonpregnant individuals has many erudite articles for and against elective sterilization by hysterectomy.

I agree that tubal sterilization is quicker and less costly and is associated with less morbidity. There is a small failure rate and the mortality rate is similar to that of hysterectomy. A certain number of patients require further gynecologic surgery in the future.

Pregnancy modifies this and makes the choice of procedure more difficult. It is disturbing that both Drs. Sloan and Priver have missed the point. They suggest an alternate procedure such as outpatient suction aspiration and laparoscopy. Seventy-seven percent of our patients were greater than 12 weeks' gestation. It is only recently that physicians have begun performing dilatation and evacuation. Prior to 1975, when this study was undertaken, only a few physicians were attempting dilatation and evacuation. Our choices then

were intra-amniotic hypertonic saline plus a laparotomy for tubal ligation or a hysterotomy with a tubal ligation.

Fortunately, other procedures have become available with a reduction in the number of hysterectomies performed. From 1968 to 1974, 500 (1.9%) hysterectomies out of a total of 25,681 abortions were performed. From 1975 to 1980, 88 (0.04%) hysterectomies out of 21,889 abortions were performed. Hysterectomy abortion has less morbidity but the costs are similar to those of hysterotomy. Hysterectomy and saline abortion with sterilization have similar rates of morbidity but hysterectomy requires an additional 1 to 2 days of hospitalization.

Dr. Priver stated that we are providing a religiously inoffensive form of sterilization to Catholic/Spanish patients. Actually a non-Catholic, non-Spanish-American patient was more likely to have sterilization performed. At least 75% of our patients are Spanish-American and Catholic while 36.8% of the study group was Spanish-American.

Dr. Sloan stated that hysterectomy in a patient less than 35 years old is indeed infrequent or even rare. Even though only 1.9% of all patients underwent hysterectomy, 18 (not 12) patients had carcinoma in situ, 82 had fibroids, and 42 had a prior cesarean section, myomectomy, or hysterotomy.

Dr. Sloan also stated, "... a 13% failure rate for tubal ligation is manyfold more than the generally accepted one." Actually our failure rate is less than 1%. The 13% refers to the number of patients of the total 500 who requested sterilization by hysterectomy after failure of tubal ligation. This was only a small percentage of the failures.

Prior to 1975, what was the procedure of choice to abort a patient greater than 12 weeks' gestation with severe cardiac disease, severe hypertension, or sickle cell disease?

For the patient who is greater than 12 weeks' gestation and requests sterilization, abortion hysterectomy is the procedure of choice if the patient has gynecologic abnormalities, prior uterine surgery, or certain medical conditions.

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# ROSTER

## Roster of American obstetrical and gynecological societies\*

(Appears annually in the January 1 issue)

**The American College of Obstetricians and Gynecologists.** (1951) *President*, Hermann Rhu. *Secretary*, William Mixson, 250 Catalonia Ave., Coral Gables, Fla. 33134. Meeting, April 26-30, 1981.

**American Gynecological Society.** (1876) *President*, J. Robert Willson. *Secretary*, A. Brian Little, Case Western Reserve University, 2065 Adelbert Rd., Cleveland, Oh. 44106.

**American Association of Obstetricians and Gynecologists.** (1888) *President*, George W. Mitchell, Jr. *Secretary*, Edgar L. Makowski, 4200 E. 9th Ave., Denver, Colo. 80262. Meetings, May 20-23, 1981, and Sept. 9-11, 1981.

**Central Association of Obstetricians and Gynecologists.** (1929) *President*, David G. Anderson. *Secretary*, George D. Malkasian, Jr., Mayo Clinic, 200 1st St., S.W., Rochester, Minn. 55901. Meeting, Oct. 15-17, 1981.

**South Atlantic Association of Obstetricians and Gynecologists.** (1938) *President*, Roy Parker. *Secretary*, F. T. Given, Jr., Ste. 208, 844 Kempsville Rd., Norfolk, Va. 23505. Meeting, Jan. 25-28, 1981.

**Alabama Association of Obstetricians and Gynecologists.**

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**Society for Gynecologic Investigation.** *President*, Pentti K. Siiteri. *Secretary*, W. Ann Reynolds, 203 Administration Bldg., 190 N. Oval Mall, Ohio State University, Columbus, Oh. 43210. Meeting, March 18-21, 1981. (By invitation only)

**Society of Obstetricians and Gynaecologists of Canada.** (1945) *President*, J. L. Vigeant. *Secretary*, J. C. G. Whetham, Ste. 406, 25 Leonard Ave., Toronto, Ont., M5T 2R2 Can. Meeting, June 16-20, 1981.

**American Board of Obstetrics and Gynecology, Inc.** (1930) *President*, William J. Dignam. *Secretary*, James A. Merrill, 711 Stanton Young Blvd., Oklahoma City, Okla. 73104. Meetings, Feb. 2-5, 1981, and Nov. 9-13, 1981.

**Pacific Coast Obstetrical and Gynecological Society.** (1931) *President*, Colin C. McCorriston. *Secretary*, David C. Figge, Ob-Gyn (RH-20), Seattle, Wash. 98195. Meeting, Sept. 27-Oct. 3, 1981.

**American Association of Gynecologic Laparoscopists.** (1972)

**Atlanta Obstetrical and Gynecological Society.** (1953) *President*, Jacob Epstein. *Secretary*, A. V. Hallum, Jr., #320, 35 Collier Rd., Atlanta, Ga. 30309. Meetings, March and Sept.

**Bronx Gynecological and Obstetrical Society.** (1924) *President*, Peter G. Mancuso. *Secretary*, Kay Anderson, 1120 Morris Park Ave., Bronx, N.Y. 10461. Meetings, third Monday, Jan.-May and Sept.-Nov.

**Brooklyn Gynecological Society, Inc.** *President*, Herbert Harris. *Secretary*, Thomas J. Manzo, Box 24, 450 Clarkson Ave., Brooklyn, N.Y. 11203. Meetings, Jan.-May and Oct.-Nov.



**Buffalo Gynecologic and Obstetric Society.** (1945) *President*, Russell J. VanCoevering. *Secretary*, Marvin J. Pleskow, 77 Livingston St., Buffalo, N.Y. 14213. Meetings, Jan. 13, Feb. 10, March 10, and April 14, 1981.

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**Cincinnati Obstetrical and Gynecological Society.** (1876)

**Connecticut Society of American Board of Obstetricians and Gynecologists, Inc.** (1952)

**East Bay Gynecological Society.** (1948) *President*, R. DeValle. *Secretary*, H. Milton Watchers, Ste. 200, 2415 High School Ave., Concord, Calif. 94520. Meetings, monthly.

**Florida Obstetric and Gynecologic Society.** *President*, Taylor H. Kirby, Jr. *Secretary*, Francis Lee Howington, P.O. Box 6339, Ft. Myers, Fla. 33901. Meeting, Dec. 3-6, 1981.

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**Indiana Obstetrical and Gynecological Society.** (1947) *Chairman*, J. Phillip Tyndall. *Secretary*, Philip N. Eskew, Jr., Ste. D., 313 E. Carmel Drive, Carmel, Ind. 46032. Meetings, third Wednesday, Jan., April, and Sept.

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**Memphis Obstetrical and Gynecological Society.** *President*, R. L. Ballenger. *Secretary*, Betty Schettler, 6025 Walnut Grove Rd., Memphis, Tenn. 38119. Meetings, six yearly.

**Miami Obstetrical and Gynecological Society.** (1946) *President*, Grover C. McDaniel. *Secretary*, Richard H. Corson, 8905 SW 87 Ave., Miami, Fla. 33156. Meetings, Jan. 8, March 12, and May 7, 1981.

**Milwaukee Gynecological Society.** (1951) *President*, Jay Larkey. *Secretary*, Robert H. Herzog, 850 Elm Grove Rd., Elm Grove, Wisc. 53122. Meetings, Feb. 9, March 9, April 13, Oct. 6, and Nov. 3, 1981.

**Montana Obstetrical and Gynecological Society.** (1948) *President*, John Heine. *Secretary*, James E. Nickel, 1930 9th Ave., Helena, Mont. 59601. Meeting, March 5-7, 1981.

**Nassau Obstetrical and Gynecological Society, Inc.** (1944) *President*, Henry W. Davoli. *Secretary*, S. Theodore Horwitz, 179 Community Dr., Great Neck, N.Y. 11020. Meetings, third Monday, Oct., Dec., Feb., and April.

**New Jersey Obstetrical and Gynecological Society.** (1947) *President*, James P. Thompson. *Secretary*, Robert L. Malatesta, Two Princess Rd., Lawrenceville, N.J. 08648.

**New York Gynecological Society, Inc.** *President*, Alfred Tanz. *Secretary*, Velimir S. Svesko, 153 W. 11th St., New York, N.Y. 10011. Meetings, Jan. 28, March 25, May 27, and Oct. 28, 1981.

**North Dakota Society of Obstetrics and Gynecology.** (1938) *President*, William Turkula. *Secretary*, S. P. Christensen, 737 Broadway, Fargo, N.D. 58102. Meeting, Sept. 11-12, 1981.

**The Obstetrical and Gynecological Society of Suffolk County.**

**Obstetrical Society of Boston.**

**The Obstetrical Society of Philadelphia.** (1868) *President*, Kaighn Smith. *Secretary*, Francis L. Hutchins, Jr., 433 Lankenau Medical Bldg., Philadelphia, Pa. 19151. Meetings, Jan. 8, Feb. 12, March 5, April 2, and May 14, 1981.

**Oklahoma City Obstetrical and Gynecological Society.** (1940) *President*, Asa Standley Porter. *Secretary*, Morris S. Curry, Ste. 530, 5100 N. Brookline, Oklahoma City, Okla. 73119. Meetings, Feb. 9, April 13, and May 11, 1981.

**Omaha Obstetrical and Gynecological Society.** (1946)

**Peninsula Gynecologic Society.** (1954) *President*, John C. Crouse. *Secretary*, Robert L. Bratman, 105 South Drive, Mountain View, Calif. 94040. Meetings, quarterly.

**Phoenix Obstetrical and Gynecological Society.** *President*, Franklin D. Loffer. *Secretary*, James R. Mouer, 2320 N. 3rd St., Phoenix, Ariz. 85004. Meetings, usually third Thursday of month.

**St. Louis Gynecological Society.** (1877) *President*, James G. Blythe. *Secretary*, Frank Ostapowicz, 3 Hill-

vale Dr., St. Louis, Mo. 63105. Meetings, second Thursday, Oct., Dec., Feb., and April.

**San Antonio Obstetrical and Gynecological Society.** (1963) *President*, Antonio Cavazos. *Secretary*, W. C. Schorlemer, 374 Metropolitan Professional Bldg., San Antonio, Tex. 78212.

**San Diego Gynecological Society.** (1937) *President*, Thomas G. Moyers. *Secretary*, Robert Resnik, Dept. Ob/Gyn, 225 Dickinson St., San Diego, Calif. 92103. Meetings, monthly.

**San Francisco Gynecological Society.** (1929) *President*, Donald Minkler. *Secretary*, Robert N. Smith, 350 Parnassus Ave., San Francisco, Calif. 94117. Meetings, second Tuesday of each month, Oct.-May.

**Shufelt Gynecologic Society of Santa Clara Valley.** (1947) *President*, Gerald E. Trobough. *Secretary*, T. J. Wright, 2504 Samaritan Dr., San Jose, Calif. 95124.

**Texas Association of Obstetricians and Gynecologists.** (1931) *President*, William H. Nash. *Secretary*, George B. Coale, 8100 Greenbriar, Houston, Tex. 77054. Meeting, March 5-7, 1981.

**Toledo Society of Obstetricians and Gynecologists.**

**Tri-Counties Obstetrical and Gynecological Society.** (1959) *President*, Richard Reisman, 2705 Loma Vista Rd., Ventura, Calif. 93003. *Secretary*, Jeffrey R. Richardson. Meetings, third Thursday, winter months.

**Virginia Obstetrical and Gynecological Society.** (1936) *President*, John Q. Hatten. *Secretary*, D. W. Branch, P.O. 8477, Roanoke, Va. 24014. Meeting, March 20-21, 1981.

**Washington Gynecological Society.** (1933) *President*, James M. Frawley. *Secretary*, John E. Jeffrey, 10031 Kendale Rd., Potomac, Md. 20854. Meetings, Jan. 14, March 11, and May, 1981.

**West Virginia Obstetrical and Gynecological Society.** (1935)

**Wisconsin Obstetrical and Gynecological Society.** *President*, Wm. Fetherston. *Secretary*, E. Howard Theis, 92 E. Division St., Fond du Lac, Wisc. 54935. Meeting, June 11-13, 1981.

**Youngstown Society of Obstetricians and Gynecologists.** *President*, Travis French. *Corresponding Secretary*, Pamela J. Stiver, P.O. Box 2125, Youngstown, Oh. 44504.



## ITEMS

### **The Central Association of Obstetricians and Gynecologists**

The Central Association of Obstetricians and Gynecologists will meet next at The Camelback Inn in Scottsdale, Arizona, on October 15, 16, and 17, 1981.

For information contact the Secretary: Dr. George D. Malkasian, Jr., Mayo Clinic, 200 First St. S. W., Rochester, Minnesota 55901.

### **Donations of excess medical and hospital equipment for Latin America and the Caribbean**

The medical and hospital equipment you often replace to upgrade services by present standards is still in great demand in poor communities of developing countries. The Pan American Development Foundation (PADF) is working to collect such equipment in the United States for clinical and health extension services in poor communities in Latin America and the Caribbean.

The Health Services Program of PADF has the cooperation of national health ministries, the Organization of American States (OAS), the Pan American Health Organization (PAHO), the American Hospital Association (AHA), and private nonprofit institutions in Latin America and the United States in bolstering health services for poor communities of the hemisphere.

PADF provides a tax receipt for all donations and coordinates inspection, shipping, and installation/training procedures.

The following types of equipment are especially needed: operating room equipment and supplies and diagnostic, therapeutic, and laboratory equipment.

All equipment should be in good working condition so that your donations may be put to use upon arrival at the clinics.

Send offers to: Program Director, Health Services Program, Pan American Development Foundation, 1625 Eye St., N.W., Suite 622, Washington, D. C. 20006.

### **Symposium on Recent Advances on Pathophysiology of Amniotic Fluid**

The Department of Obstetrics and Gynaecology, University of Modena, under the auspices of the Italian Society of Obstetrics and Gynaecology announces a

symposium, "Recent Advances on Pathophysiology of Amniotic Fluid," to be held March 27-28, 1981, at the Centro Congressi Hotel Fini, Modena, Italy.

### **Hawaii Imaging Conference**

The American College of Medical Imaging announces the "Hawaii Imaging Conference," on ultrasound, computed tomography, and nuclear medicine, to be held April 11-18, 1981, at the Kauai Surf Hotel, Island of Kauai, Hawaii.

For further information write: American College of Medical Imaging, P. O. Box 27188, Los Angeles, California 90027.

### **Fourth Annual Mid-Winter Symposium in Obstetrics and Gynecology**

The Fourth Annual Mid-Winter Symposium in Obstetrics and Gynecology will be held February 25-27, 1981, at the Scottsdale Hilton Hotel, Scottsdale, Arizona. It is cosponsored by the Phoenix Obstetrics and Gynecology Society and the Department of Obstetrics and Gynecology, Maricopa County General Hospital.

For information contact: The Secretary, Phoenix OB/GYN Society, 333 E. Virginia, Suite 222, Phoenix, Arizona 85004.

### **Medical meetings—1981**

1. Pan-American Conference on Fertility and Sterility, February 12-20, 1981, Mullet Bay, St. Maarten, N.A.
2. American College of Osteopathic Obstetricians and Gynecologists, February 14-21, 1981, Puerto Vallarta, Mexico
3. The First International Convention on Medico-Legal Aspects of Disability, February 15-19, 1981, Tel Aviv, Israel
4. Third World Congress of Human Reproduction, March 22-26, 1981, Berlin, West Germany
5. The Fifth World Congress of Sexology, June 21-26, 1981, Jerusalem, Israel
6. Second International Congress of Andrology, June 28-30, 1981, Tel Aviv, Israel

7. Second International Dermatopathology Symposium, July 12-15, 1981, London, England

8. The Sixth International Congress of Human Genetics, September 13-18, 1981, Jerusalem, Israel

For a brochure please write or call: Medical Congress Coordinators, 1212 Avenue of the Americas, New York, New York 10036. Telephone: (212) 840-0110.

#### **Forty-eighth Annual Convention of The American College of Osteopathic Obstetricians and Gynecologists**

The Forty-eighth Annual Convention of The American College of Osteopathic Obstetricians and Gynecologists, "Practical Primary Care for Women," will be held at the Dunes Hotel, Las Vegas, Nevada, February 9-13, 1981.

For scientific and travel information, contact the officially appointed agent for the United States: Medical Congress Coordinators, 1212 Avenue of the Americas, New York, New York 10036.

#### **The Foundation Prize Thesis**

The American Association of Obstetricians and Gynecologists Foundation, Incorporated, annually solicits manuscripts in competition for a prize award of \$1,500.00. An anonymous committee appointed by the President of the Foundation will review the papers and select the winning paper or recommend no award if none meets the Foundation's standards. Competitors for this award are subject to the following: Eligible contestants shall include: (1) interns, residents, or graduate students in obstetrics-gynecology; (2) persons with an M.D. degree, or a scientific degree approved by the Prize Award Committee, who are actively practicing or teaching obstetrics-gynecology or engaged in clinical investigation or research in these or closely allied fields. No Fellow of the American Association of Obstetricians and Gynecologists or the American Gynecological Society shall be eligible to compete for this award, as either author or co-author. The contestant must have done the major share of the work and the writing of the Prize Paper. Manuscripts must not exceed 5,000 words and must be typewritten, double-spaced, and on one side of the sheet. Manuscripts must be presented under a nom de plume which shall in no way indicate the author's identity. Three copies of all manuscripts and illustrations entered in a given year must be in the hands of the President of the Foundation by March 31, 1981. The award will be given at the May, 1981, Annual Meeting of the American Association, at which time the successful contestant must appear in person to present the thesis as a part of the regular scientific program, in conformity with the rules of the Association. The Foundation will pay the travel and subsistence costs for that meeting for the awardee and spouse.

Inquiries must be addressed to the Secretary for detailed information: Abe Mickal, M.D., Secretary, AAOG Foundation, Louisiana State University, School of Medicine, 1542 Tulane Ave., New Orleans, Louisiana 70112.

#### **Pan-American Conference on Fertility and Sterility**

The Pan-American Conference on Fertility and Sterility will be held at the Mullet Bay Beach Hotel, St. Maarten, Netherlands, Antilles, February 12-20, 1981. A Pre-Conference Post Graduate Course will be presented February 12, 1981.

For scientific details and information contact: U. S. International Foundation for Studies in Reproduction, Inc., 112-44 69th Ave., Forest Hills, New York 11375.

For information on travel and accommodations contact: Medical Congress Coordinators, 1212 Avenue of the Americas, New York, New York 10036.

#### **Alternative Aspects of Prepared Childbirth Education**

The Division of Gynecology and Obstetrics of The Johns Hopkins Medical Institutions is presenting a nursing seminar, entitled "Alternative Aspects of Prepared Childbirth Education," February 25, 1981, in the Turner Auditorium of The Johns Hopkins Medical Institutions, Baltimore, Maryland.

For further information contact: Program Coordinator, The Johns Hopkins University, Turner 22, 720 Rutland Ave., Baltimore, Maryland 21205.

#### **First Symposium on Computers in Perinatal Medicine**

"The First Symposium on Computers in Perinatal Medicine," sponsored by the Perinatal Clinical Research Center at Cleveland Metropolitan General Hospital/Case Western Reserve University, will be held May 18-20, 1981, in Cleveland, Ohio. The program is designed for obstetricians, pediatricians, epidemiologists, and biomedical engineers. Abstracts on the following topics should be submitted by January 15, 1981: Computerized Perinatal Information Management, including Data Acquisition—Patient Charts/Forms Design, Use of Patient Information—Risk Assessment, Database Management of Perinatal Information and Computers in Perinatal Research, including Electronic Fetal Monitoring, Statistical Data Analysis, Neonatal Intensive Care Systems, and other related topics. Papers accepted with manuscripts submitted in their entirety will be published in the *Proceedings* of the Symposium.

For author and registration information, contact: Lawrence Chik, Ph.D., Co-Director, Biomedical Engineering, Perinatal Clinical Research Center, Cleveland Metropolitan General Hospital, 3395 Scranton Road, Cleveland, Ohio 44109.



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# INDEX TO ADVERTISERS

|                                                                                                      |                           |                                                                          |                        |
|------------------------------------------------------------------------------------------------------|---------------------------|--------------------------------------------------------------------------|------------------------|
| <b>Ayerst Laboratories</b><br><i>Premarin</i> -----                                                  | 16, 17, 18                | <b>Medical University of South Carolina</b><br><i>Fellowships</i> -----  | 30                     |
| <b>Baystate Medical Center</b><br><i>Opportunity Available</i> -----                                 | 30                        | <b>Merrell</b><br><i>Yutopar</i> -----                                   | 33, 34, 35, 36, 37, 38 |
| <b>Burroughs Wellcome Co.</b><br><i>Empirin c Codeine #3</i> -----                                   | 4                         | <b>Prime Health</b><br><i>Opportunity Available</i> -----                | 28                     |
| <b>College of Physicians and Surgeons of Columbia University</b><br><i>Postgraduate Course</i> ----- | 30                        | <b>Purdue Frederick Company, The</b><br><i>Senokot-S</i> -----           | 8                      |
| <b>Dorsey Laboratories</b><br><i>Bellergal-S</i> -----                                               | Third Cover, Fourth Cover | <b>Searle Laboratories</b><br><i>Demulen</i> -----                       | 12, 13, 14             |
| <b>Duke University</b><br><i>Opportunities Available</i> -----                                       | Third Cover               | <b>Syntex</b><br><i>Norinyl 1+35</i> -----                               | Second Cover, 1, 2     |
| <b>Fancee Free</b><br><i>Maternity Apparel</i> -----                                                 | 28                        | <b>Temple University Hospital</b><br><i>Opportunity Available</i> -----  | 28                     |
| <b>Kauai Medical Group, Inc.</b><br><i>Opportunity Available</i> -----                               | 30                        | <b>Thomas, Charles C</b><br><i>Books</i> -----                           | 20                     |
| <b>3M</b><br><i>Precise Disposable Skin Stapler</i> -----                                            | 26, 27                    | <b>University of South Alabama</b><br><i>Opportunity Available</i> ----- | 28                     |
| <i>Steri-Strip Skin Closures</i> -----                                                               | 3                         | <b>Wyeth Laboratories</b><br><i>Lo/Oural</i> -----                       | 22, 23, 24             |
| <b>McGraw-Hill Book Co.</b><br><i>Text-Reference</i> -----                                           | 29                        | <b>Zeiss, Inc., Carl</b><br><i>OpMi 6-S Microscope</i> -----             | 6                      |
| <b>Mead Johnson Pharmaceutical Division</b><br><i>Ovcon-35</i> -----                                 | 31, 32                    |                                                                          |                        |

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# *American Journal of* OBSTETRICS AND GYNECOLOGY

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2. She wants nonhormonal contraception.
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Please see next page for a brief summary of product information.



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**Description:** The Cu-7 has coiled around the vertical limb 89 mg. of copper wire providing approximately 200 mm<sup>2</sup> of exposed copper surface area. The Tatum-T has coiled around the vertical limb 120 mg. of copper wire providing approximately 210 mm<sup>2</sup> of exposed copper surface area.

**Contraindications:** Not to be inserted when any of the following conditions exist: pregnancy or suspicion of pregnancy; abnormalities of the uterus resulting in distortion of the uterine cavity; acute pelvic inflammatory disease or history of repeated pelvic inflammatory disease; postpartum endometritis or infected abortion in the past three months; known or suspected uterine or cervical disease such as hyperplasia or carcinoma including unresolved, abnormal "Pap" smear; vaginal bleeding of unknown etiology; untreated acute cervicitis until infection is controlled; diagnosed Wilson's disease; known or suspected allergy to copper; previous ectopic pregnancy; significant anemia; and valvular heart disease, leukemia, or use of chronic corticosteroid therapy.

### Warnings

1. **Pregnancy:** (a) **Long-term effects:** The long-term effects on the offspring of the presence of copper in the uterus when pregnancy occurs are unknown.

(b) **Septic abortion:** Reports have indicated an increased incidence of septic abortion associated in some instances with septicemia, septic shock and death in patients becoming pregnant with an IUD in place. Most of these reports have been associated with the mid-trimester of pregnancy. In some cases, the initial symptoms have been insidious and not easily recognized. If pregnancy should occur with a Cu-7 or Tatum-T in situ, the IUD should be removed if the thread is visible or, if removal proves to be or would be difficult, interruption of the pregnancy should be considered and offered to the patient as an option, bearing in mind that the risks associated with an elective abortion increase with gestational age.

(c) **Continuation of pregnancy:** If the patient chooses to continue the pregnancy and the Cu-7 or Tatum-T remains in situ, she must be warned of increased risk of spontaneous abortion and increased risk of sepsis, including death. The patient must be closely observed and advised to report immediately all abnormal symptoms, such as flu-like syndrome, fever, abdominal cramping and pain, bleeding, or vaginal discharge, because generalized symptoms of septicemia may be insidious.

2. **Ectopic pregnancy:** (a) Pregnancy which occurs with an IUD in situ is more likely to be ectopic than pregnancy occurring without an IUD. Therefore, patients who become pregnant while using a Cu-7 or Tatum-T should be carefully evaluated for the possibility of ectopic pregnancy.

(b) Special attention should be directed to patients with delayed menses, slight metrorrhagia and/or unilateral pelvic pain, and to those patients who wish to interrupt a pregnancy occurring in the presence of a Cu-7 or Tatum-T, to determine whether ectopic pregnancy has occurred.

3. **Pelvic infection:** An increased risk of pelvic inflammatory disease associated with the use of IUDs has been reported. While unconfirmed, this risk appears to be greatest for young women who are nulliparous and/or who have multiple sexual partners. Salpingitis can result in tubal damage and occlusion, thereby threatening future fertility. Therefore, it is recommended that patients be taught to look for symptoms of pelvic inflammatory disease. The decision to use an IUD in a particular case must be made by the physician and patient with consideration of a possible deleterious effect on future fertility.

Pelvic infection may occur with a Cu-7 or Tatum-T in situ and at times result in the development of tubo-ovarian abscesses or general peritonitis. The symptoms of pelvic infection include: new development of menstrual disorders (prolonged or heavy bleeding), abnormal vaginal discharge, abdominal or pelvic pain, dyspareunia, fever. The symptoms are especially significant if they occur following the first few cycles after insertion. Appropriate aerobic and anaerobic bacteriologic studies should be done and antibiotic therapy initiated promptly. If the infection does not show marked clinical improvement within 24 to 48 hours, the Cu-7 or Tatum-T should be removed and continuing treatment reassessed on the basis of results of culture and sensitivity tests.

4. **Embedment:** Partial penetration or lodging of the Cu-7 or Tatum-T in the endometrium or myometrium can result in difficult removal. This may occur more frequently in smaller uteri. See removal instructions.

5. **Perforation:** Partial or total perforation of the uterine wall or cervix may occur, usually during insertions into patients sooner than two months after abortion or delivery, or in uterine cavities too small. The possibility of perforation must be kept in mind during insertion and at the time of any subsequent examination. If perforation occurs, laparotomy or laparoscopy should be performed as soon as medically feasible and the Cu-7 or Tatum-T removed. Abdominal adhesions, intestinal penetration, intestinal obstruction, and local inflammatory reaction with abscess formation and erosion of adjacent viscera may result if the Cu-7 or Tatum-T is left in the peritoneal cavity.

6. **Medical diathermy:** The use of medical diathermy (short-wave and microwave) in a patient with a metal-containing IUD may cause heat injury to surrounding tissue. Therefore, medical diathermy to the abdominal and sacral areas should not be used on patients using a Cu-7 or Tatum-T.

7. **Effects of copper:** Additional amounts of copper available to the body from the Cu-7 or Tatum-T may precipitate symptoms in women with undiagnosed Wilson's disease. The incidence of Wilson's disease is 1 in 200,000.

### Precautions

1. **Patient counseling:** Prior to insertion the physician, nurse, or other trained health professional must provide the patient with the Patient Brochure. The patient should be given the opportunity to read the brochure and discuss fully any questions she may have concerning the Cu-7 or Tatum-T as well as other methods of contraception.

2. **Patient evaluation and clinical considerations:** (a) A complete medical history should be obtained to determine conditions that might influence the selection of an IUD. A physical examination should include a pelvic examination, "Pap" smear, gonorrhea culture and, if indicated, appropriate tests for other forms of venereal disease. The physician should determine that the patient is not pregnant.

(b) The uterus should be carefully sounded prior to insertion to determine the degree of patency of the endocervical canal and internal os, and the direction and depth of the uterine

cavity. Exercise care to avoid perforation with the sound. DO NOT USE THE INSERTION INSTRUMENT AS A SOUND. In occasional cases, severe cervical stenosis may be encountered. Do not use excessive force to overcome this resistance.

(c) The uterus usually sounds to a depth of 6 to 8 cm. Insertion into a uterine cavity measuring less than 6.5 cm. by sounding may increase the incidence of pain, bleeding, partial or complete expulsion, perforation, and possibly pregnancy.

(d) To reduce the possibility of insertion in the presence of existing undetermined pregnancy, the optimal time for insertion is the latter part of the menstrual flow or one or two days thereafter. The Cu-7 or Tatum-T should not be inserted post partum or post abortion until involution of the uterus is complete. The incidence of perforation and expulsion is greater if involution is not complete.

It is, however, necessary to place the Cu-7 or Tatum-T as high as possible within the uterine cavity to help avoid partial or complete expulsion that could result in pregnancy.

Physicians are cautioned that it is imperative for them to become thoroughly familiar with the instructions for use before attempting placement of the Cu-7 or Tatum-T.

(e) IUDs should be used with caution in those patients who have anemia or a history of menorrhagia or hypermenorrhea. Patients experiencing menorrhagia and/or metrorrhagia following IUD insertion may be at risk for the development of hypochromic microcytic anemia. Also, IUDs should be used with caution in patients receiving anticoagulants or having a coagulopathy.

(f) Syncope, bradycardia or other neurovascular episodes may occur during insertion or removal of IUDs, especially in patients with a previous disposition to these conditions.

(g) Patients with valvular or congenital heart disease are more prone to develop subacute bacterial endocarditis than patients who do not have such disease. Use of an IUD in these patients may represent a potential source of septic emboli.

(h) Use of an IUD in patients with acute cervicitis should be postponed until proper treatment has cleared up the infection.

(i) Since the Cu-7 or Tatum-T may be partially or completely expelled, patients should be reexamined and evaluated shortly after the first postinsertion menses, but definitely within three months after insertion. Thereafter annual examination with appropriate medical and laboratory evaluation should be carried out. The Cu-7 or Tatum-T should be replaced every three years.

(j) The patient should be told that some bleeding or cramping may occur during the first few weeks after insertion, but if these symptoms continue or are severe she should report them to her physician. She should be instructed on how to check after each menstrual period to make certain that the thread still protrudes from the cervix and cautioned that there is no contraceptive protection if the Cu-7 or Tatum-T has been expelled. She should also be cautioned not to dislodge the Cu-7 or Tatum-T by pulling on the thread. If a partial expulsion occurs, removal is indicated and a new Cu-7 or Tatum-T may be inserted. The patient should be told to return within three years for removal of the Cu-7 or Tatum-T and for replacement if desired.

(k) A copper-induced urticarial allergic skin reaction may develop in women using a copper-containing IUD. If symptoms of such an allergic response occur, the patient should be instructed to tell the consulting physician that a copper-containing device is being used.

(l) The Cu-7 or Tatum-T should be removed for the following medical reasons: menorrhagia and/or metrorrhagia producing significant anemia; uncontrolled pelvic infection; intractable pain often aggravated by intercourse, dyspareunia; pregnancy, if the thread is visible; endometrial or cervical malignancy; uterine or cervical perforation; or any indication of partial expulsion.

(m) If the retrieval thread cannot be seen it may have retracted into the uterus or have been broken off, or the Cu-7 or Tatum-T may have been expelled. Localization usually can be made by feeling with a probe; if not, x-ray or sonography can be used. When the physician elects to recover a Cu-7 or Tatum-T with the thread not visible, the removal instructions should be considered.

(n) If any patient with a Cu-7 or Tatum-T suddenly develops overt clinical hepatitis or abnormal liver function tests, appropriate diagnostic procedures should be initiated.

**Adverse Reactions:** Perforations of uterus and cervix have occurred. Perforation into the abdomen has been followed by abdominal adhesions, intestinal penetration, intestinal obstruction, local inflammatory reaction with abscess formation and erosion of adjacent viscera. Pregnancy has occurred with the Cu-7 or Tatum-T in situ and when either has been partially or completely expelled.

The incidence of spontaneous abortion, when conception occurs with intrauterine devices in situ, appears to be increased over that in unprotected women. Insertion cramping, usually of no more than a few seconds' duration, may occur; however, some women may experience residual cramping for several hours or even days. Intermenstrual spotting or bleeding or prolonged or increased menstrual flow may occur.

Pelvic infection including salpingitis with tubal damage or occlusion has been reported. This may result in future infertility. Complete or partial expulsion of the Cu-7 or Tatum-T may sometimes occur, particularly in those patients with uteri measuring less than 6.5 cm. by sounding. Urticarial allergic skin reaction may occur. The following complaints have also been reported with IUDs although their relation to the Cu-7 or Tatum-T has not been established: amenorrhea or delayed menses, backaches, cervical erosion, cystic masses in the pelvis, vaginitis, leg pain or soreness, weight loss or gain, nervousness, dyspareunia, cystitis, endometritis, septic abortion, septicemia, leukorrhea, ectopic pregnancy, difficult removal, uterine embedment, anemia, pain, neurovascular episodes including bradycardia and syncope secondary to insertion, dysmenorrhea, and fragmentation of the IUD.

**Clinical Efficacy:** In clinical trials, use effectiveness was determined as follows for parous and nulliparous women, as tabulated by the life table method. (Rates are expressed as cumulative events per 100 women through 12, 24, and 36 months of use.)

Cu-7 experience encompasses 387,131 woman-months of use, including 12 months for 11,517 women, 24 months for 7,895, and 36 months for 3,661.

Tatum-T experience encompasses 236,060 woman-months of use in the United States and Canada, including 12 months for 8,232 women, 24 months for 4,247, and 36 months for 1,408.

Cumulative pregnancy rates were:

|         | 12 Months |             | 24 Months |             | 36 Months |             |
|---------|-----------|-------------|-----------|-------------|-----------|-------------|
|         | Parous    | Nulliparous | Parous    | Nulliparous | Parous    | Nulliparous |
| Cu-7    | 1.9       | 1.7         | 2.9       | 2.6         | 3.4       | 3.4         |
| Tatum-T | 3.0       | 2.1         | 4.9       | 4.5         | 6.0       | 5.8         |

Please see complete product labeling for additional information, including insertion and removal instructions.

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OT1-2



Proven highly effective for the  
treatment of serious infections\*

# Mefoxin<sup>®</sup> IV/IM

(Cefoxitin Sodium | MSD)

VIALS, containing 1 gram and 2 grams cefoxitin equivalent

\*Due to susceptible strains of indicated  
bacteria at indicated sites.

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# Now Indicated for prophylaxis to reduce the incidence of certain postoperative infections complicating

• GI surgery<sup>†</sup> • Vaginal hysterectomy<sup>†</sup> • Cesarean section<sup>‡</sup>

Providing a broad spectrum—including *Bacteroides fragilis*

In controlled clinical trials, MEFOXIN<sup>®</sup> (Cefoxitin Sodium, MSD)  
reduced the incidence of certain postoperative infections  
with use limited to a 24-hour period following the operative procedure.

Prophylactic administration should usually be stopped within 24 hours since continuing administration of any antibiotic increases the possibility of adverse reactions.

**† Perioperatively:** Two grams administered intravenously or intramuscularly just prior to surgery (approximately ½ to 1 hour before the initial incision); then 2 grams every 6 hours for no more than 24 hours.

**‡ Cesarean-section patients:** The first dose of 2 grams is administered intravenously as soon as the umbilical cord is clamped. The second and third doses should be given as 2 grams intravenously or intramuscularly 4 hours and 8 hours after the first dose. Subsequent doses may be given every 6 hours for no more than 24 hours.

For complete details on dosage and administration, see full prescribing information.

If there are signs of infection, specimens for

of the causative organisms so that appropriate therapy may be instituted.

MEFOXIN (Cefoxitin Sodium, MSD) is contraindicated in patients who have shown hypersensitivity to cefoxitin and the cephalosporin group of antibiotics. Before therapy with MEFOXIN is instituted, careful inquiry should be made to determine whether the patient has had previous hypersensitivity reactions to cefoxitin, cephalosporins, penicillins, or other drugs. This product should be given with caution to penicillin-sensitive patients. Antibiotics should be administered with caution to any patient who has demonstrated some form of allergy, particularly to drugs. If an allergic reaction to MEFOXIN occurs, discontinue the drug. Serious hypersensitivity reactions may require epinephrine and other emergency measures. Use of the drug in women of childbearing potential requires that the anticipated benefit be weighed against the possible risks.

For a brief summary of prescribing information,

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# Mefoxin<sup>®</sup> IV/IM

(Cefoxitin Sodium | MSD)

**Indications and Usage: Treatment**—Serious infections caused by susceptible strains of the designated microorganisms in the following diseases:

**LOWER RESPIRATORY TRACT INFECTIONS**, including pneumonia and lung abscess, caused by *Streptococcus pneumoniae* (formerly *Diplococcus pneumoniae*), other streptococci (excluding enterococci, e.g., *Strep. faecalis*), *Staphylococcus aureus* (penicillinase and non-penicillinase producing), *Escherichia coli*, *Klebsiella* species, *Hemophilus influenzae*, and *Bacteroides* species.

**GENITOURINARY INFECTIONS**. Urinary tract infections caused by *E. coli*, *Klebsiella* species, *Proteus mirabilis*, indole-positive *Proteus* (i.e., *P. morganii*, *P. rettgeri*, and *P. vulgaris*), and *Providencia* species. Uncomplicated gonorrhea due to *Neisseria gonorrhoeae*.

**INTRA-ABDOMINAL INFECTIONS**, including peritonitis and intra-abdominal abscess, caused by *E. coli*, *Klebsiella* species, *Bacteroides* species including the *B. fragilis* group,<sup>‡</sup> and *Clostridium* species. **GYNECOLOGICAL INFECTIONS**, including endometritis, pelvic cellulitis, and pelvic inflammatory disease, caused by *E. coli*, *N. gonorrhoeae*, *Bacteroides* species including the *B. fragilis* group,<sup>‡</sup> *Clostridium* species, *Peptococcus* species, *Peptostreptococcus* species, and group B streptococci.

**SEPTICEMIA** caused by *Strep. pneumoniae* (formerly *D. pneumoniae*), *Staph. aureus* (penicillinase and non-penicillinase producing), *E. coli*, *Klebsiella* species, and *Bacteroides* species including the *B. fragilis* group.<sup>‡</sup>

**BONE AND JOINT INFECTIONS** caused by *Staph. aureus* (penicillinase and non-penicillinase producing).

**SKIN AND SKIN STRUCTURE INFECTIONS** caused by *Staph. aureus* (penicillinase and non-penicillinase producing), *Staph. epidermidis*, streptococci (excluding enterococci, e.g., *Strep. faecalis*), *E. coli*, *P. mirabilis*, *Klebsiella* species, *Bacteroides* species including the *B. fragilis* group,<sup>‡</sup> *Clostridium* species, *Peptococcus* species, and *Peptostreptococcus* species.

Although appropriate culture and susceptibility studies should be performed, therapy may be started while awaiting these results. Cefoxitin is not active *in vitro* against most strains of *Pseudomonas aeruginosa* and enterococci (e.g., *Strep. faecalis*) and many strains of *Enterobacter cloacae*. Methicillin-resistant staphylococci are almost uniformly resistant to cefoxitin.

**Prevention**—Prophylactic use perioperatively (preoperatively, intraoperatively, and postoperatively) in surgical procedures (e.g., vaginal hysterectomy, gastrointestinal surgery) classified as contaminated or potentially contaminated or in patients in whom infection at the operative site would present a serious risk, e.g., prosthetic arthroplasty; intraoperatively (after umbilical cord is clamped) and postoperatively in cesarean section.

MEFOXIN usually should be given ½ to 1 hour before the operation, which is sufficient time to achieve effective levels in the wound during the procedure. Prophylactic administration should usually be stopped within 24 hours since continuing administration of any antibiotic increases the possibility of adverse reactions but, in the majority of surgical procedures, does not reduce the incidence of subsequent infection. However, in patients undergoing prosthetic arthroplasty, it is recommended that MEFOXIN be continued for 72 hours after the surgical procedure. If there are signs of infection, specimens for culture should be obtained for the identification of the causative organism so that appropriate therapy may be instituted.

**Contraindications**: Previous hypersensitivity to cefoxitin and the cephalosporin group of antibiotics.

**Warnings**: BEFORE THERAPY IS INSTITUTED, CAREFUL INQUIRY SHOULD BE MADE TO DETERMINE PREVIOUS HYPERSENSITIVITY REACTIONS TO CEFOXITIN, CEPHALOSPORINS, PENICILLINS, OR

OTHER DRUGS. GIVE WITH CAUTION TO PENICILLIN-SENSITIVE PATIENTS. ANTIBIOTICS SHOULD BE ADMINISTERED WITH CAUTION TO ANY PATIENT WHO HAS DEMONSTRATED SOME FORM OF ALLERGY, PARTICULARLY TO DRUGS. IF AN ALLERGIC REACTION TO CEFOXITIN OCCURS, DISCONTINUE THE DRUG. SERIOUS HYPERSENSITIVITY REACTIONS MAY REQUIRE EPINEPHRINE AND OTHER EMERGENCY MEASURES.

**Precautions**: The total daily dose should be reduced in patients with transient or persistent reduction of urinary output due to renal insufficiency because high and prolonged serum antibiotic concentrations can occur in such individuals from usual doses. As with other antibiotics, prolonged use may result in overgrowth of nonsusceptible organisms; repeated evaluation of the patient's condition is essential. If superinfection occurs, take appropriate measures. Increased nephrotoxicity has been reported following concomitant administration of cephalosporins and aminoglycoside antibiotics.

**Interference with Laboratory Tests**—As with cephalothin, high concentrations (> 100 mcg/ml) may interfere with measurement of serum and urine creatinine levels by the Jaffé reaction and produce false increases of modest degree in creatinine levels reported; serum samples should not be analyzed for creatinine if withdrawn within 2 hours of cefoxitin administration. A false-positive reaction for glucose in urine has been observed with CLINITEST<sup>®</sup> reagent tablets.

**Pregnancy**—In women of childbearing potential, weigh anticipated benefit against possible risks.

**Nursing Mothers**—Cefoxitin is excreted in human milk in low concentrations.

**Infants and Children**—Safety and efficacy in infants from birth to three months have not yet been established. In children three months and older, higher doses have been associated with increased incidence of eosinophilia and elevated SGOT.

**Adverse Reactions**: The most common adverse reactions have been local reactions following intravenous or intramuscular injection. Other adverse reactions have been encountered infrequently. **Local Reactions**—Thrombophlebitis with intravenous administration; pain, induration, and tenderness after intramuscular injections. **Allergic Reactions**—Rash, pruritus, eosinophilia, fever, and other allergic reactions. **Gastrointestinal**—Nausea, vomiting, and diarrhea. **Blood**—Transient eosinophilia, leukopenia, neutropenia, and hemolytic anemia; a positive direct Coombs test may develop in some individuals, especially those with azotemia. **Liver Function**—Transient elevations in SGOT, SGPT, serum LDH, and serum alkaline phosphatase. **Renal Function**—Elevations in serum creatinine and/or blood urea nitrogen levels.

**Note**: In group A beta-hemolytic streptococcal infections, therapy should be maintained for at least 10 days to guard against the risk of rheumatic fever or glomerulonephritis. In staphylococcal and other infections involving a collection of pus, surgical drainage should be carried out where indicated. Intramuscular injections should be well within the body of a relatively large muscle such as the upper outer quadrant of the buttock (i.e., gluteus maximus); aspiration is necessary to avoid inadvertent injection into a blood vessel. The total daily dosage in infants and children should not exceed 12 grams.

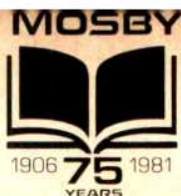
**How Supplied**: Sterile cefoxitin sodium in vials and infusion bottles containing 1 gram or 2 grams cefoxitin equivalent.

For more detailed information, consult your MSD representative or see full prescribing information. Merck Sharp & Dohme, Division of Merck & Co., Inc., West Point, PA 19486

<sup>‡</sup>*B. fragilis*, *B. distasonis*, *B. ovatus*, *B. thetaiotaomicron*, *B. vulgatus*  
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# American Journal of Obstetrics and Gynecology

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## Contents

January 15

1981

### Clinical opinion

#### Evaluation of elective repeat cesarean section as a standard of care: An application of decision analysis

123

Kirkwood K. Shy, M.D., M.P.H., James P. LoGerfo, M.D., M.P.H., and  
Laurence E. Karp, M.D.  
Seattle, Washington

### Gynecology

#### Sexually transmitted conditions among women college students

130

William M. McCormack, M.D., John R. Evrard, M.D., Catherine F. Laughlin,  
Bernard Rosner, Ph.D., Susan Alpert, M.S., Vicki Ann Crockett, Dorothy McComb, M.S., and  
Stephen H. Zinner, M.D.  
Boston, Massachusetts, and Providence, Rhode Island

(Contents continued on page 7)

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## Contents continued from page 5

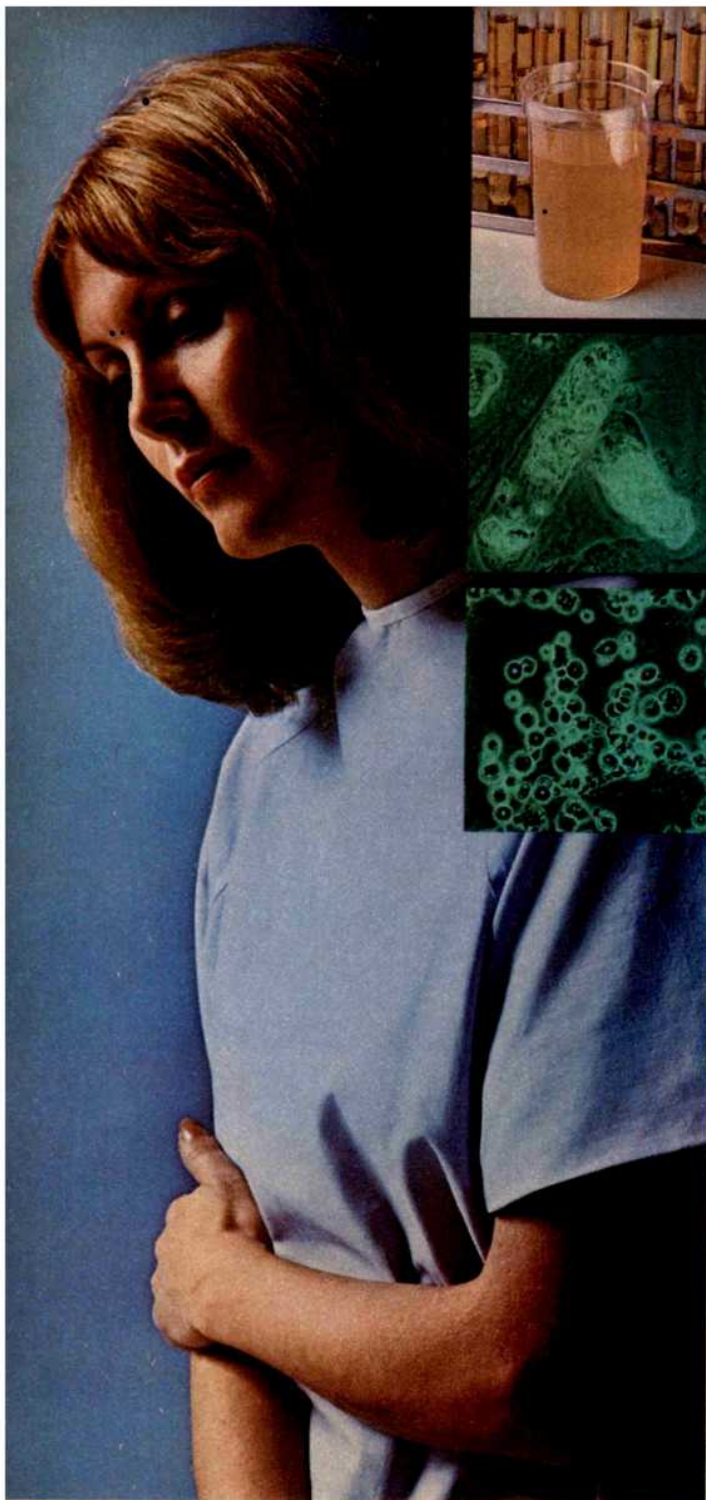
- Mechanism of luteolysis: Effect of estradiol and prostaglandin  $F_{2\alpha}$  on corpus luteum luteinizing hormone/human chorionic gonadotropin receptors and cyclic nucleotides in the rhesus monkey** 134  
Ginter Sotrel, M.D., Ahmet Helvacioğlu, M.D., Susan Dowers, B.S., Antonio Scommegna, M.D., and Frederick J. Auletta, Ph.D.  
*Chicago, Illinois*
- Deaths associated with laparoscopic sterilization by unipolar electrocoagulating devices, 1978 and 1979** 141  
Herbert B. Peterson, M.D., Howard W. Ory, M.D., Joel R. Greenspan, M.D., and Carl W. Tyler, Jr., M.D.  
*Atlanta, Georgia*
- Cryosurgical treatment of cervical intraepithelial neoplasia with follow-up of five to eight years** 144  
Erik Hemmingsson, M.D., Ulf Stendahl, M.D., and Stig Stensón, M.D.  
*Uppsala, Sweden*
- Fine-needle aspiration cytology in the management of gynecologic cancer** 148  
Jerome L. Belinson, M.D., Jean M. Lynn, R.N., Jacalyn L. Papillo, C.T.(A.S.C.P.), Kenneth Lee, M.D., and Roy Korson, M.D.  
*Burlington, Vermont*
- The significance of atypical vessels and neovascularization in cervical neoplasia** 154  
Frederick Sillman, M.D., John Boyce, M.D., and Rachel Fruchter, Ph.D.  
*Brooklyn, New York*
- Effect of cyproheptadine on chlorpromazine stimulation of prolactin in women** 160  
John A. Board, M.D., and Robert J. Fierro, M.D.  
*Richmond, Virginia*
- Progesterone-induced smooth muscle-like cells in the subperitoneal nodules produced by estrogen** 164  
Shingo Fujii, M.D., Noboru Nakashima, M.D., Hitoshi Okamura, M.D., Akira Takenaka, M.D., Hideharu Kanzaki, M.D., Yuji Okuda, M.D., Kohki Morimoto, M.D., and Toshio Nishimura, M.D.  
*Kyoto, Japan*

## Obstetrics

- Gestational age assessment. I. Analysis of individual clinical observations** 173  
H. Frank Andersen, M.D., Timothy R. B. Johnson, Jr., M.D., Mel L. Barclay, M.D., and Jairus D. Flora, Jr., Ph.D.  
*Ann Arbor, Michigan*

(Contents continued on page 9)





In acute UTI

When your first choice  
is Macrochantin®  
you'll rarely need  
a second one

**Macrochantin is potent against  
both major uropathogens**

in office practice—*E. coli*, the most common, and  
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antimicrobials used individually or in combination.  
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97%...against enterococcus, 94%.

**Superior in vitro\* against the two most common uropathogens**

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**TMP/SMX 95%**

**Sulfonamide 73%**

*Enterococcus*

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**TMP/SMX 51%**

**Sulfonamide 6%**

\*In vitro data do not necessarily predict clinical efficacy.

1. PMR Bacteriologic Report, Summer Series 1979; a national bacteriologic  
monitoring service for 200 acute-care hospitals of 100 beds or more.

...too valuable to keep in reserve

**Macrochantin®** Capsules: 25, 50, 100mg  
(nitrofurantoin macrocrystals)

**INDICATIONS:** Macrochantin is indicated for the treatment of urinary tract infections when due to susceptible strains of *E. coli*, enterococci, *S. aureus* (it is not indicated for the treatment of associated renal cortical or perinephric abscesses), and certain susceptible strains of *Klebsiella* species, *Enterobacter* species, and *Proteus* species.

**NOTE:** Specimens for culture and susceptibility testing should be obtained prior to and during drug administration.

**CONTRAINDICATIONS:** Anuria, oliguria, or significant impairment of renal function (creatinine clearance under 40 ml per minute) are contraindications. Treatment of this type of patient carries an increased risk of toxicity because of impaired excretion of the drug. For the same reason, this drug is much less effective under these circumstances.

Contraindicated in pregnant patients at term as well as in infants under one month of age because of the possibility of hemolytic anemia due to immature enzyme systems (glutathione instability), and those known to have a hypersensitivity to Macrochantin, Furadantin® (nitrofurantoin), and other nitrofurantoin preparations.

**WARNINGS:** Acute, subacute and chronic pulmonary reactions have been observed. If these reactions occur, the drug should be withdrawn and appropriate measures taken.

An insidious onset of pulmonary reactions (diffuse interstitial pneumonitis or pulmonary fibrosis, or both) in patients on long-term therapy warrants close monitoring.

There have been isolated reports giving pulmonary reactions as a contributing cause of death. (See Hypersensitivity reactions.)

Cases of hemolytic anemia of the primaquine sensitivity type have been induced. The hemolysis appears to be linked to a glucose-6-phosphate dehydrogenase deficiency in the red blood cells of the affected patients. This deficiency is found in 10 percent of Negroes and a small percentage of ethnic groups of Mediterranean and Near-Eastern origin. Any sign of hemolysis is an indication to discontinue the drug. Hemolysis ceases when the drug is withdrawn.

*Pseudomonas* is the organism most commonly implicated in superinfections in patients treated with Macrochantin.

Hepatitis, including chronic active hepatitis, has been observed rarely. Fatalities have been reported. The mechanism appears to be of an idiosyncratic hypersensitive type.

**PRECAUTIONS:** Peripheral neuropathy may occur. This may become severe or irreversible. Fatalities have been reported. Predisposing conditions, renal impairment (creatinine clearance under 40 ml per minute), anemia, diabetes, electrolyte imbalance, vitamin B deficiency, and debilitating disease may enhance such occurrence.

**Usage in Pregnancy:** Safety during pregnancy and lactation has not been established. Use of this drug in women of childbearing potential requires that the anticipated benefit be weighed against the possible risks.

**ADVERSE REACTIONS:** Gastrointestinal reactions: Anorexia, nausea and emesis are the most frequent reactions; abdominal pain and diarrhea occur less frequently. These dose-related toxicity reactions can be minimized by reduction of dosage, especially in the female patient. Hepatitis occurs rarely.

**Hypersensitivity reactions:** Pulmonary sensitivity reactions may occur, which can be acute, subacute, or chronic.

Acute reactions are commonly manifested by fever, chills, cough, chest pain, dyspnea, pulmonary infiltration with consolidation or pleural effusion on x-ray, and eosinophilia. They usually occur within the first week of treatment and are reversible with cessation of therapy. Resolution may be dramatic.

In subacute reactions, fever and eosinophilia are observed less often. Recovery is somewhat slower, perhaps as long as several months. If the symptoms are not recognized as being drug related and nitrofurantoin is not withdrawn, symptoms may become more severe.

Chronic pulmonary reactions are more likely to occur in patients who have been on continuous therapy for six months or longer. Insidious onset of malaise, dyspnea on exertion, cough, and altered pulmonary function are common manifestations. Roentgenographic and histologic findings of diffuse interstitial pneumonitis or fibrosis, or both, are also common manifestations. Fever is rarely prominent. The severity of these reactions and the degree of their resolution appear to be related to the duration of therapy after the first clinical signs appear. Pulmonary function may be permanently impaired even after cessation of nitrofurantoin therapy. This risk is greater when pulmonary reactions are not recognized early.

**Dermatologic reactions:** Maculopapular, erythematous, or eczematous eruption, pruritus, urticaria, and angioedema.

**Other hypersensitivity reactions:** Anaphylaxis, asthmatic attack in patients with history of asthma, cholestatic jaundice, hepatitis, including chronic active hepatitis, drug fever, and arthralgia.

**Hematologic reactions:** Hemolytic anemia, granulocytopenia, leukopenia, eosinophilia, and megaloblastic anemia. Return of the blood picture to normal has followed cessation of therapy.

**Neurologic reactions:** Peripheral neuropathy, headache, dizziness, nystagmus, and drowsiness.

**Miscellaneous reactions:** Transient alopecia. As with other antimicrobial agents, superinfections by resistant organisms may occur. With Macrochantin, however, these are limited to the genitourinary tract because suppression of normal bacterial flora elsewhere in the body does not occur.

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## Contents continued from page 7

---

- The effect of diazepam on rates of fertilization in the CF-1 mouse** 178  
R. A. Williamson, M.D., and M. A. Stenchever, M.D.  
*Seattle, Washington*
- Effects of vasoactive polypeptides on the uterine vasculature** 182  
Kenneth E. Clark, Ph.D., E. Gay Mills, B.S., Stanley J. Stys, M.D., and Asa E. Seeds, M.D.  
*Cincinnati, Ohio*

## Fetus, placenta, and newborn

---

- Effects of maternal acetonuria and low pregnancy weight gain on children's psychomotor development** 189  
Richard L. Naeye, M.D., and Ronald A. Chez, M.D.  
*Hershey, Pennsylvania*
- Antepartum fetal heart rate testing. VII. The significance of fetal bradycardia** 194  
Maurice L. Druzin, M.B., B.Ch., José Gratacós, M.D., Kirk A. Keegan, M.D., and Richard H. Paul, M.D.  
*Los Angeles, California*
- Evaluation of the Corometrics 315 telemetry system for fetal monitoring** 199  
Jeffrey Lipshitz, M.B., Ch.B., M.R.C.O.G., F.A.C.O.G., James E. Wade, Garland D. Anderson, M.D., F.A.C.O.G., and Baha M. Sibai, M.D.  
*Memphis, Tennessee*
- Catecholamines in human amniotic fluid** 204  
M. Phillippe, M.D., and K. J. Ryan, M.D.  
*Boston, Massachusetts*
- Comparison of mercury levels in maternal blood, fetal cord blood, and placental tissues** 209  
Paul M. Kuhnert, Ph.D., Betty R. Kuhnert, Ph.D., and Penny Erhard, B.S.  
*Cleveland, Ohio*
- The effect of cervical/vaginal secretions on measurements of lecithin/sphingomyelin ratio and optical density at 650 nm** 214  
A. J. Sbarra, G. Blake, C. L. Cetrulo, R. J. Selvaraj, M. J. Herschel, C. Delise, J. L. Kennedy, and G. W. Mitchell, Jr.  
*Boston, Massachusetts*

## Communications in brief

---

- Gastrointestinal hemorrhage in puerperium of pre-eclamptic patients who received glucocorticoid therapy** 217  
Stefan Semchyshyn, M.D.  
*Park Ridge, Illinois*

(Contents continued on page 11)



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## Contents continued from page 9

|                                                                                                                                  |     |
|----------------------------------------------------------------------------------------------------------------------------------|-----|
| <b>Gonococcal peritonitis following uterine manipulation at laparoscopy</b>                                                      | 218 |
| Joel E. Goodnough, M.D., Richard O'Shaughnessy, M.D., and Dennis Shoff, M.D.<br><i>Columbus, Ohio</i>                            |     |
| <b>Detrusor muscle instability—A complication of surgery?</b>                                                                    | 219 |
| G. J. Jarvis, F.R.C.S., M.R.C.O.G.<br><i>Sheffield, England</i>                                                                  |     |
| <b>Solitary paraganglioma of the vagina—Report of a case</b>                                                                     | 219 |
| G. Pezeshkpour, M.D., F.C.A.P.<br><i>Lewes, Delaware</i>                                                                         |     |
| <b>Unsuspected trisomy 18: A case for an examination protocol in stillborn infants</b>                                           | 221 |
| Suzanne B. Cassidy, M.D., Thomas J. Benedetti, M.D., and Virginia P. Sybert, M.D.<br><i>Seattle, Washington</i>                  |     |
| <b>Uterus didelphys unicollis with adenocarcinoma in one horn and atypical endometrial hyperplasia in the other: Case report</b> | 222 |
| Eduard Eichner, M.D., and Kathleen A. Simak, M.D.<br><i>Cleveland, Ohio</i>                                                      |     |

## Correspondence

|                       |     |
|-----------------------|-----|
| <b>Correspondence</b> | 226 |
|-----------------------|-----|

## Books

|                       |     |
|-----------------------|-----|
| <b>Books received</b> | 238 |
|-----------------------|-----|

*Information for authors on page 21*

*Index to advertisers on page 44*

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Serious as well as minor side effects have been reported with the use of oral contraceptives. These include thromboembolic disease. The physician should remain alert to the earliest manifestations of any symptoms of serious disease and discontinue oral contraceptive therapy when appropriate. The physician should be fully aware of the Complete Prescribing Information for this product.

**Please see Complete Prescribing Information, a brief summary of which appears on the next page.**

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**IMPORTANT NOTE:** This information is a brief summary of the complete prescribing information prepared by the manufacturer and should not be used as the basis for prescribing the product. This summary was prepared by deleting from the complete prescribing information certain text, tables, and references. The physician should be thoroughly familiar with the complete prescribing information and patient information before prescribing the product.

**INDICATION: CONTRACEPTION.** The pregnancy rate in women using conventional combination oral contraceptives (containing 35 mcg or more of ethinyl estradiol or 50 mcg or more of mestranol) is generally reported as less than one pregnancy per 100 woman-years of use. Slightly higher rates (slightly more than one pregnancy per 100 woman-years of use) are reported for some combination products containing 35 mcg or less of ethinyl estradiol, and rates on the order of three pregnancies per 100 woman-years are reported for the progestogen-only oral contraceptives. Table 1 gives ranges of pregnancy rates reported in the literature for other means of contraception. The efficacy of these means of contraception (except the IUD) depends upon the degree of adherence to the method.

**Table 1. Pregnancy Rates per 100 Women-Years:** IUD, less than 1-6; Diaphragm with spermicidal product (creams or jellies), 2-20; Condom, 3-36; Aerosol foams, 2-29; Jellies and creams, 4-36; Periodic abstinence (rhythm) all types, less than 1-47; 1. Calendar method, 14-47; 2. Temperature method, 1-20; 3. Temperature method-intercourse only in postovulatory phase, less than 1-7; 4. Mucus method, 1-25; No contraception, 60-80. **DOSE-RELATED RISK OF THROMBOEMBOLISM FROM ORAL CONTRACEPTIVES:** Two studies have shown a positive association between the dose of estrogens in oral contraceptives and the risk of thromboembolism. For this reason, it is prudent and in keeping with good principles of therapeutics to minimize exposure to estrogen. The oral contraceptive product prescribed for any given patient should be that product which contains the least amount of estrogen that is compatible with an acceptable pregnancy rate and patient acceptance. It is recommended that new acceptors of oral contraceptives be started on preparations containing 0.05 mg or less of estrogen.

**CONTRAINDICATIONS:** Oral contraceptives should not be used in women with any of the following conditions: 1. Thrombophlebitis or thromboembolic disorders. 2. A past history of deep vein thrombophlebitis or thromboembolic disorders. 3. Cerebral vascular or coronary artery disease. 4. Known or suspected carcinoma of the breast. 5. Known or suspected estrogen-dependent neoplasia. 6. Undiagnosed abnormal genital bleeding. 7. Known or suspected pregnancy (see WARNINGS, No. 5). 8. Benign or malignant liver tumor which developed during the use of oral contraceptives or other estrogen-containing products.

#### WARNINGS

**Cigarette smoking increases the risk of serious cardiovascular side effects from oral contraceptive use. This risk increases with age and with heavy smoking (15 or more cigarettes per day) and is quite marked in women over 35 years of age. Women who use oral contraceptives should be strongly advised not to smoke.**

**The use of oral contraceptives is associated with increased risk of several serious conditions including thromboembolism, stroke, myocardial infarction, hepatic adenoma, gallbladder disease, hypertension. Practitioners prescribing oral contraceptives should be familiar with the following information relating to these risks.**

1. **THROMBOEMBOLIC DISORDERS AND OTHER VASCULAR PROBLEMS.** An increased risk of thromboembolic and thrombotic disease associated with the use of oral contraceptives is well established. Four principal studies in Great Britain and three in the United States have demonstrated an increased risk of fatal and nonfatal venous thromboembolism and stroke, both hemorrhagic and thrombotic. These studies estimate that users of oral contraceptives are 4 to 11 times more likely than nonusers to develop these diseases without evident cause. Overall excess mortality due to pulmonary embolism or stroke is on the order of 1.0 to 3.5 deaths annually per 100,000 users and increases with age. **CEREBROVASCULAR DISORDERS:** In a collaborative American study of cerebrovascular disorders in women with and without predisposing conditions, it was estimated that the risk of hemorrhagic stroke was 2.0 times greater in users than in nonusers and their risk of thrombotic stroke was 4.0 to 9.5 times greater in users than in nonusers.

**MYOCARDIAL INFARCTION:** An increased risk of myocardial infarction associated with the use of oral contraceptives has been reported confirming a previously suspected association. These studies, conducted in the United Kingdom, found, as expected, that the greater the number of underlying risk factors for coronary artery disease (cigarette smoking; hypertension, hypercholesterolemia, obesity, diabetes, history of pre-eclamptic toxemia), the higher the risk of developing myocardial infarction, regardless of whether the patient was an oral contraceptive user or not. Oral contraceptives, however, were found to be a clear additional risk factor. The annual excess case rate (increased risk) of myocardial infarction (fatal and nonfatal) in oral contraceptive users was estimated to be approximately 7 cases per 100,000 women users in the 30-39 age group and 67 cases per 100,000 women users in the 40-44 age group. In terms of relative risk, it has been estimated that oral contraceptive users who do not smoke (smoking is considered a major predisposing condition to myocardial infarction) are about twice as likely to have a fatal myocardial infarction as nonusers who do not smoke. Oral contraceptive users who are also smokers have about a 5-fold increased risk of fatal infarction compared to users who do not smoke, but about a 10- to 12-fold increased risk compared to nonusers who do not smoke. Furthermore, the amount of smoking is also an important factor. In determining the importance of these relative risks, however, the baseline rates for various age groups must be given serious consideration. The importance of other predisposing conditions mentioned above in determining relative and absolute risks has not as yet been quantified, it is quite likely that the same synergistic action exists, but perhaps to a lesser extent. **Risk of Dose:** In an analysis of data derived from several national adverse reaction reporting systems, British investigators concluded that the risk of thromboembolism including coronary thrombosis is directly related to the dose of estrogen used in oral contraceptives. Preparations containing 100 mcg or more of estrogen were associated with a higher risk of thromboembolism than those containing 50-80 mcg of estrogen. Their analysis did suggest, however, that the quantity of estrogen may not be the sole factor involved. This finding has been confirmed in the United States. Careful epidemiological studies to determine the degree of thromboembolic risk associated with progestogen-only oral contraceptives have not been performed. Cases of thromboembolic disease have been reported in women using these products, and they should not be presumed to be free of excess risk. The risk of thromboembolic and thrombotic disorders, in both users and nonusers of oral contraceptives, increases with age. Oral contraceptives are, however, an independent risk factor for these events. **ESTIMATE OF EXCESS MORTALITY FROM CIRCULATORY DISEASES:** A large prospective study carried out in the United Kingdom estimated the mortality rate per 100,000 women per year from diseases of the circulatory system for users and nonusers of oral contraceptives according to age, smoking habits, and duration of use. The overall excess death rate annually from circulatory diseases for oral contraceptive users was estimated to be 20 per 100,000 (ages 15-34-5/100,000; ages 35-44-33/100,000; ages 45-49-140/100,000), the risk being concentrated in older women, in those with a long duration of use, and in cigarette smokers. It was not possible, however, to examine the interrelationships of age, smoking, and duration of use, nor to compare the effects of continuous versus intermittent use. Although the study showed a 10-fold increase in death due to circulatory diseases in users for five or more years, all of these deaths occurred in women 35 or older. Until larger numbers of women under 35 with continuous use for five or more years are available, it is not possible to assess the magnitude of the relative risk for this younger age group. Another study published at the same time confirms a previously reported increase of mortality in pill users from cardiovascular disease. The available data from a variety of sources have been analyzed to estimate the risk of death associated with various methods of contraception. The estimates of risk of death for each method include the combined risk of the contraceptive method (e.g., thromboembolic and thrombotic disease in the case of oral contraceptives) and the risk attributable to pregnancy or abortion in the event of method failure. This latter risk varies with the effectiveness of the contraceptive method. The findings of this analysis are shown in Figure 1 below. The study concluded that the mortality associated with all methods of birth control is low and below that associated with childbirth, with the exception of oral contraceptives in women over 40 who smoke. (The rates given for pill only smokers for each age group are for smokers as a class. For "heavy" smokers [more than 15 cigarettes a day], the rates given would be about double, for "light" smokers [less than 15 cigarettes a day], about 50 percent.) The mortality associated with oral contraceptive use in non-smokers over 40 is higher than with any other method of contraception in that age group. The lowest mortality is associated with the condom or diaphragm backed up by early abortion. The risk of thromboembolic and thrombotic disease associated with oral contraceptives increases with age after approximately age 30 and, for myocardial infarction, is further increased by hypertension, hypercholesterolemia, obesity, diabetes, or history of pre-eclamptic toxemia and especially by cigarette smoking. The risk of myocardial infarction in oral contraceptive users is substantially increased in women age 40 and over, especially those with other risk factors. Based on the data currently available, the following chart gives a gross estimate of the risk of death from circulatory disorders associated with the use of oral contraceptives.

#### SMOKING HABITS AND OTHER PREDISPOSING CONDITIONS—RISK ASSOCIATED WITH USE OF ORAL CONTRACEPTIVES

| Age                                         | Below 30 | 30-39 | 40+ |
|---------------------------------------------|----------|-------|-----|
| Heavy smokers                               | C        | B     | A   |
| Light smokers                               | D        | C     | B   |
| Non-smokers (no predisposing conditions)    | D        | C,D   | C   |
| Non-smokers (other predisposing conditions) | C        | C,B   | B,A |

The physician and the patient should be alert to the earliest manifestations of thromboembolic and thrombotic disorders (e.g., thrombophlebitis, pulmonary embolism, cerebrovascular insufficiency, coronary occlusion, retinal thrombosis, and mesenteric thrombosis). Should any of these occur or be suspected, the drug should be discontinued immediately. A four- to six-fold increased risk of postsurgery thromboembolic complications has been reported in oral contraceptive users. If feasible, oral contraceptives should be discontinued at least four weeks before surgery of a type associated with an increased risk of thromboembolism or prolonged immobilization. 2. **OCULAR LESIONS:** There have been reports of neuro-ocular lesions such as optic neuritis or retinal thrombosis associated with the use of oral contraceptives. Discontinue oral contraceptive medication if there is unexplained, sudden or gradual, partial or complete loss of vision; onset of proptosis or diplopia; papilledema; or retinal vascular lesions and institute appropriate diagnostic and therapeutic measures. 3. **CARCINOMA:** Long-term continuous administration of either natural or synthetic estrogen in certain animal species increases the frequency of carcinoma of the breast, cervix, vagina, and liver. Certain synthetic progestogens, none currently contained in oral contraceptives, have been noted to increase the incidence of mammary nodules, benign and malignant, in dogs. In humans, three case control studies have reported an increased risk of endometrial carcinoma associated with the prolonged use of exogenous estrogen in postmenopausal women. One publication reported on the first 21 cases submitted by physicians to a registry of cases of adenocarcinoma of the endometrium in women under 40 on oral contraceptives. Of the cases found in women without predisposing risk factors for adenocarcinoma of the endometrium (e.g., irregular bleeding at the time oral contraceptives were first given, polycystic ovaries), nearly all occurred in women who had used a sequential oral contraceptive. These products are no longer marketed. No evidence has been reported suggesting an increased risk of endometrial cancer in users of conventional combination or progestogen-only oral contraceptives. Several studies have found no increase in breast cancer in women taking oral contraceptives or estrogens. One study, however, while also noting no overall increased risk of breast cancer in women treated with oral contraceptives, found an excess risk in the subgroups of oral contraceptive users with documented benign breast disease. A reduced occurrence of benign breast tumors in users of oral contraceptives has been well-documented. In summary, there is at present no confirmed evidence from human studies of an increased risk of cancer associated with oral contraceptives. Close clinical surveillance of all women taking oral contraceptives is, nevertheless, essential. In all cases of undiagnosed persistent or recurrent abnormal vaginal bleeding, appropriate diagnostic measures should be taken to rule out malignancy. Women with a strong family history of breast cancer or who have breast nodules, fibrocystic disease or abnormal mammograms should be monitored with particular care if they elect to use oral contraceptives instead of other methods of contraception.

|                                        | 15-19 | 20-24 | 25-29 | 30-34 | 35-39 | 40-44 |
|----------------------------------------|-------|-------|-------|-------|-------|-------|
| No method                              | 5.5   | 5.2   | 7.1   | 14.0  | 19.3  | 21.9  |
| Abortion only                          | 2.3   | 2.5   | 2.5   | 5.2   | 9.8   | 6.6   |
| Pill only-nonsmokers                   | 1.3   | 1.4   | 1.4   | 2.2   | 4.5   | 3.1   |
| Pill only-smokers                      | 1.5   | 1.6   | 1.6   | 1.8   | 13.4  | 58.9  |
| IUDs only                              | 1.1   | 1.2   | 1.2   | 1.4   | 1.6   | 1.4   |
| Traditional contraception only         | 1.1   | 1.4   | 1.9   | 3.7   | 4.7   | 4.0   |
| Traditional contraception and abortion | 0.3   | 0.4   | 0.4   | 0.8   | 1.4   | 0.8   |

4. **HEPATIC TUMORS.** Benign hepatic adenomas have been found to be associated with the use of oral contraceptives. One study showed that oral contraceptive formulations with high hormonal potency were associated with a higher risk than lower potency formulations and use of oral contraceptives with high hormonal potency and age over 30 years may further increase the woman's risk of hepatocellular adenoma. Although benign, hepatic adenomas may rupture and may cause death through intra-abdominal hemorrhage. This has been reported in short-term as well as long-term users of oral contraceptives. Two studies relate risk with duration of use of the contraceptive, the risk being much greater after four or more years of oral contraceptive use. While hepatic adenoma is a rare lesion, it should be considered in women presenting abdominal pain and tenderness, abdominal mass or shock. A few cases of hepatocellular carcinoma have been reported in women taking oral contraceptives. The relationship of these drugs to this type of malignancy is not known at this time. 5. **USE IN OR IMMEDIATELY PRECEDING PREGNANCY, BIRTH DEFECTS IN OFFSPRING, AND MALIGNANCY IN FEMALE OFFSPRING.** The use of female sex hormones—both estrogenic and progestational agents—during early pregnancy may seriously damage the offspring. It has been shown that females exposed in utero to diethylstilbestrol, a nonsteroidal estrogen, have an increased risk of developing in later life a form of vaginal or cervical cancer that is ordinarily extremely rare. This risk has been estimated to be on the order of 1 to 4 in 1000 exposures. Although there is no evidence at this time that oral contraceptives further enhance the risk of developing this type of malignancy, such patients should be monitored with particular care if they elect to use oral contraceptives instead of other methods of contraception. Furthermore, a high percentage of such exposed women (from 30 to 90%) have been found to have epithelial changes of the vagina and cervix. Although these changes are histologically benign, it is not known whether this condition is a precursor of vaginal malignancy. Male children so exposed may develop abnormalities of the urogenital tract. Although similar data are not available with the use of other estrogens, it cannot be presumed that they would not induce similar changes. An increased risk of congenital anomalies, including heart defects and limb defects, has been reported with the use of sex hormones, including oral contraceptives, in pregnancy. One case control study has estimated a 4.7-fold increase in risk of limb-reduction defects in infants exposed in utero to sex hormones (oral contraceptives, hormonal withdrawal tests for pregnancy or attempted treatment for threatened abortion). Some of these exposures were very short and involved only a few days of treatment. The data suggest that the risk of limb-reduction defects in exposed fetuses is somewhat less than one in 1000 live births. In the past, female sex hormones have been used during pregnancy in an attempt to treat threatened or habitual abortion. There is considerable evidence that estrogens are ineffective for these indications, and there is no evidence from well-controlled studies that progestogens are effective for these uses. There is some evidence that triploidy and possibly other types of polyploidy are increased among abortuses from women who become pregnant soon after ceasing oral contraceptives. Embryos with these anomalies are virtually always aborted spontaneously. Whether there is an overall increase in spontaneous abortion of pregnancies conceived soon after stopping oral contraceptives is unknown. It is recommended that for any patient who has missed two consecutive periods, pregnancy should be ruled out before continuing the contraceptive regimen. If the patient has not adhered to the prescribed schedule, the possibility of pregnancy should be considered at the time of the first missed period (or after 45 days from the last menstrual period if the progestogen-only oral contraceptives are used), and further use of oral contraceptives should be withheld until pregnancy has been ruled out. If pregnancy is confirmed, the patient should be apprised of the potential risks to the fetus and the advisability of continuation of the pregnancy should be discussed in the light of these risks. It is also recommended that women who discontinue oral contraceptives with the intent of becoming pregnant use an alternate form of contraception for a period of time before attempting to conceive. Many clinicians recommend three months although no precise information is available on which to base this recommendation. The administration of progestogen-only or progestogen-estrogen combinations to induce withdrawal bleeding should not be used as a test of pregnancy. 6. **GALLBLADDER DISEASE.** Studies report an increased risk of surgically confirmed gallbladder disease in users of oral contraceptives and estrogens. In one study, an increased risk appeared after two years of use and doubled after four or five years of use. In one of the other studies, an increased risk was apparent between six and twelve months of use. 7. **CARBOHYDRATE AND LIPID METABOLIC EFFECTS.** A decrease in glucose tolerance has been observed in a significant percentage of patients on oral contraceptives. For this reason, diabetic and diabetic patients should be carefully observed while receiving oral contraceptives. An increase in triglycerides and total phospholipids has been observed in patients receiving oral contraceptives. The clinical significance of this finding remains to be defined. 8. **ELEVATED BLOOD PRESSURE.** An increase in blood pressure has been reported in patients receiving oral contraceptives. In some women hypertension may occur within a few months of beginning oral contraceptive use. In the first year of use, the prevalence of women with hypertension is low in users and may be no higher than that of a comparable group of nonusers. The prevalence in users increases, however, with longer exposure, and in the fifth year of use is two and a half to three times the reported prevalence in the first year. Age is also strongly correlated with the development of hypertension in oral contraceptive users. Women who previously have had hypertension during pregnancy may be more likely to develop elevation of blood pressure when given oral contraceptives. Hypertension that develops as a result of taking oral contraceptives usually returns to normal after discontinuing the drug. 9. **HEADACHE:** The onset or exacerbation of migraine or development of headache of a new pattern which is recurrent, persistent, or severe, requires discontinuation of oral contraceptives and evaluation of the cause. 10. **BLEEDING IRREGULARITIES:** Breakthrough bleeding, spotting, and amenorrhea are frequent reasons for patients discontinuing oral contraceptives. In breakthrough bleeding, as in all cases of irregular bleeding from the vagina, nonfunctional causes should be borne in mind. In undiagnosed persistent or recurrent abnormal bleeding from the vagina, adequate diagnostic measures are indicated to rule out pregnancy or malignancy. If pathology has been excluded, time or a change to another formulation may solve the problem. Changing to an oral contraceptive with a higher estrogen content, while potentially useful in minimizing menstrual irregularity, should be done only if necessary since this may increase the risk of thromboembolic disease. Women with a past history of oligomenorrhea or secondary amenorrhea or young women without regular cycles may have a tendency to remain anovulatory or to become amenorrheic after discontinuation of oral contraceptives. Women with these preexisting problems should be advised of this possibility and encouraged to use other contraceptive methods. Postuse anovulation, possibly prolonged, may also occur in women without previous irregularities. 11. **ECTOPIC PREGNANCY:** Ectopic as well as intrauterine pregnancy may occur in contraceptive failures. 12. **BREAST FEEDING:** Oral contraceptives given in the postpartum period may interfere with lactation. There may be a decrease in the quantity and quality of the breast milk. Furthermore, a small fraction of the hormonal agents in oral contraceptives has been identified in the milk of mothers receiving these drugs. The effects, if any, on the breast-fed child have not been determined. If feasible, the use of oral contraceptives should be deferred until the infant has been weaned. **PRECAUTIONS:** General: 1. A complete medical and family history should be taken prior to the initiation of oral contraceptives. The pretreatment and periodic physical examinations should include special reference to blood pressure, breasts, abdomen and pelvic organs, including Papanicolaou smear and relevant laboratory tests. As a general rule, oral contraceptives should not be prescribed for longer than one year without another physical examination being performed. 2. Under the influence of estrogen-progestogen preparations, preexisting uterine leiomyomata may increase in size. 3. Patients with a history of psychic depression should be carefully observed and the drug discontinued if depression recurs to a serious degree. Patients becoming significantly depressed while taking oral contraceptives should stop the medication and use an alternate method of contraception in an attempt to determine whether the symptom is drug-related. 4. Oral contraceptives may cause some degree of fluid retention. They should be prescribed with caution, and only with careful monitoring, in patients with conditions which might be aggravated by fluid retention, such as convulsive disorders, migraine syndrome, asthma, or cardiac or renal insufficiency. 5. Patients with a past history of jaundice during pregnancy have an increased risk of recurrence of jaundice while receiving oral contraceptive therapy. If jaundice develops in any patient receiving such drugs, the medication should be discontinued. 6. Steroid hormones may be poorly metabolized in patients with impaired liver function and should be administered with caution in such patients. 7. Oral contraceptive users may have disturbances in normal tryptophan metabolism which may result in a relative pyridoxine deficiency. The clinical significance of this is yet to be determined. 8. Serum folate levels may be depressed by oral contraceptive therapy. Since the pregnant woman is predisposed to the development of folate deficiency and the incidence of folate deficiency increases with increasing gestation, it is possible that if a woman becomes pregnant shortly after stopping oral contraceptives, she may have a greater chance of developing folate deficiency and complications attributed to this deficiency. 9. The pathologist should be advised of oral contraceptive therapy when relevant specimens are submitted. 10. Certain endocrine and liver function tests and blood components may be affected by estrogen-containing oral contraceptives: a. Increased sulfobromophthalein retention. b. Increased prothrombin and factors VII, VIII, IX, and X. c. Increased antithrombin. d. Increased norepinephrine-induced platelet aggregability. e. Increased thyroid-binding globulin (TBG) leading to increased circulating free thyroid hormone, as measured by protein-bound iodine (PBI), T4 by column, or T4 by radioimmunoassay. Free T3 resin uptake is decreased, reflecting the elevated TBG. Free T4 concentration is unaltered. f. Decreased pregnanediol excretion. g. Reduced response to metyrapone test. **INFORMATION FOR THE PATIENT:** (See Patient Package Insert). **DRUG INTERACTIONS:** Reduced efficacy and increased incidence of breakthrough bleeding have been associated with concomitant use of rifampin. A similar association has been suggested with barbiturates, phenylbutazone, phenytoin sodium, ampicillin, and tetracycline. **CARCINOGENESIS, PREGNANCY, NURSING MOTHERS:** See CONTRAINDICATIONS and WARNINGS. **ADVERSE REACTIONS:** An increased risk of the following serious adverse reactions has been associated with the use of oral contraceptives (see WARNINGS): Thrombophlebitis. Pulmonary embolism. Coronary thrombosis. Cerebral thrombosis. Cerebral hemorrhage. Hypertension. Gallbladder disease. Liver tumors. Congenital anomalies. There is evidence of an association between the following conditions and the use of oral contraceptives, although additional confirmatory studies are needed: Mesenteric thrombosis. Neuro-ocular lesions, e.g., retinal thrombosis and optic neuritis. The following adverse reactions have been reported in patients receiving oral contraceptives and are believed to be drug-related: Nausea, usually the most common adverse reaction. Vomiting, occurs in approximately 10% or less of patients during the first cycle. Other reactions, as a general rule, are seen much less frequently or only occasionally. Gastrointestinal symptoms (such as abdominal cramps and bloating). Breakthrough bleeding. Spotting. Change in menstrual flow. Dysmenorrhea. Amenorrhea during and after treatment. Temporary infertility after discontinuance of treatment. Edema. Chloasma or melasma which may persist. Breast changes: tenderness, enlargement, and secretion. Change in weight (increase or decrease). Change in cervical erosion and cervical secretion. Possible diminution in lactation when given immediately postpartum. Cholestatic jaundice. Migraine. Increase in size of uterine leiomyomata. Rash (allergic). Mental depression. Reduced tolerance to carbohydrates. Vaginal candidiasis. Change in corneal curvature (steepening). Intolerance to contact lenses. The following adverse reactions have been reported in users of oral contraceptives, although the association has been neither confirmed nor refuted: Premenstrual-like syndrome. Cataracts. Changes in libido. Chorea. Changes in appetite. Cystitis-like syndrome. Headache. Nervousness. Dizziness. Hirsutism. Loss of scalp hair. Erythema multiforme. Erythema nodosum. Hemorrhagic eruption. Vaginitis. Porphyria. Impaired renal function. **ACUTE OVERDOSE:** Serious ill effects have not been reported following acute ingestion of large doses of oral contraceptives by young children. Overdose may cause nausea, and withdrawal bleeding may occur in females.

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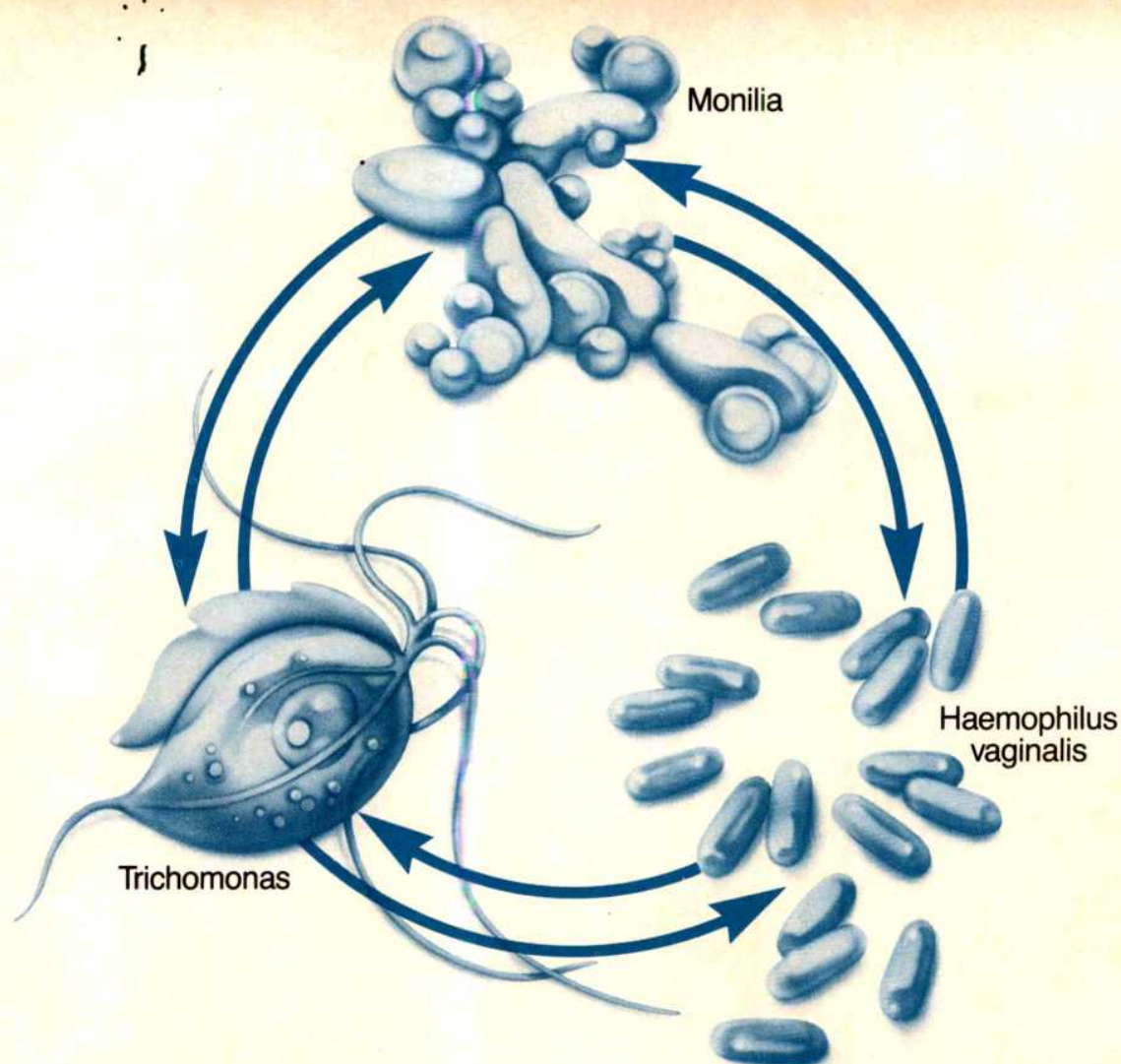
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# Triple target therapy for infectious vaginitis

## **BETADINE<sup>®</sup>**

### Microbicides Vaginitis Regimen

- Broad-spectrum microbicidal action kills all three pathogens usually responsible for infectious vaginitis—monilia, *Trichomonas* and *Haemophilus vaginalis*.
- Microbicidal action is maintained in the presence of blood, serum and vaginal secretions.
- Virtually nonirritating to skin and vaginal mucosa—nonstaining to skin and natural fabrics.



**Easy-to-follow regimen**

**IN THE OFFICE**

**BETADINE® Solution**

**AT HOME**

**BETADINE® Vaginal Gel\***

(povidone-iodine)

**BETADINE® Douche**

- In the office, swab the cervix and vulvovaginal area with BETADINE Solution.
- Prescribe BETADINE Vaginal Gel (povidone-iodine), one applicatorful of which is to be inserted each night.
- Followed the next morning by BETADINE Douche in therapeutic concentration—2 tablespoonfuls to a quart of lukewarm water.
- After two weeks
  - Patient returns for an office visit.
  - In more resistant cases, the regimen may be continued through 1 or 2 cycles, if necessary.

**\*BETADINE® Vaginal Gel**

(povidone-iodine)

**Brief Summary**

For use in the treatment of *Trichomonas vaginalis* vaginitis, monilial vaginitis and nonspecific vaginitis.

Based on a review of this drug by the National Academy of Sciences—National Research Council and/or other information, FDA has classified the indications as follows: possibly effective for use in the management of *trichomonas vaginalis* vaginitis, monilial vaginitis and non-specific vaginitis. Final classification of the less-than-effective indications requires further investigation.

**Precaution**

If irritation, redness or swelling develops, discontinue use. The rare individual with history of iodine sensitization should not use this product, pending further investigation.

**Purdue Frederick**

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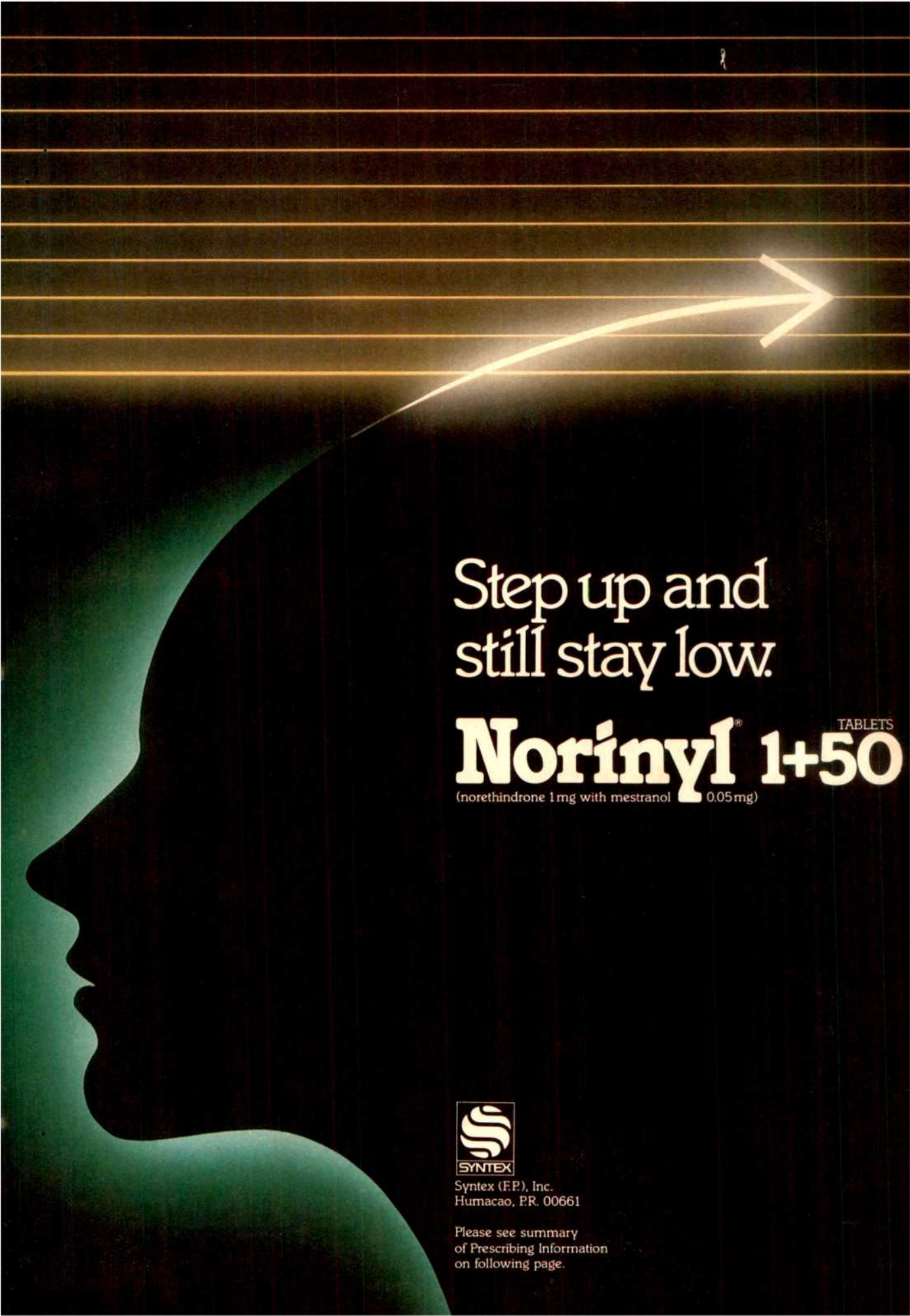
## American Journal of Obstetrics and Gynecology

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## ORAL CONTRACEPTIVE (O.C.) AGENTS

**INDICATIONS** O.C.s are indicated for the prevention of pregnancy. **DOSE-RELATED RISK OF THROMBOEMBOLISM FROM O.C.s.** Studies have shown a positive association between the dose of estrogens in O.C.s and the risk of thromboembolism. For this reason, it is prudent and in keeping with good principles of therapeutics to minimize exposure to estrogen. The O.C. product prescribed for any given patient should be that product which contains the least amount of estrogen that is compatible with an acceptable pregnancy rate and patient acceptance. It is recommended that new acceptors of O.C.s should be started on preparations containing 0.05 mg or less of estrogen.

**CONTRAINDICATIONS** 1. Known or suspected pregnancy (see Warning No. 5). 2. Thrombophlebitis or thromboembolic disorders. 3. A past history of deep vein thrombophlebitis or thromboembolic disorders. 4. Undiagnosed abnormal genital bleeding. 5. O.C.s should not be used by women who have or have had any of the following conditions: a. Cerebral vascular or coronary artery disease. b. Known or suspected carcinoma of the breast. c. Known or suspected estrogen dependent neoplasia. d. Benign or malignant liver tumor which developed during the use of oral contraceptives or other estrogen containing products.

**WARNINGS:** Cigarette smoking increases the risk of serious cardiovascular side effects from O.C.s. This risk increases with age and with heavy smoking (15 or more cigarettes per day) and is quite marked in women over 35 years of age. Women who use O.C.s should be strongly advised not to smoke.

The use of O.C.s is associated with increased risk of several serious conditions including thromboembolism, stroke, myocardial infarction, liver tumor, gall bladder disease, visual disturbances, fetal abnormalities, and hypertension. Practitioners prescribing O.C.s should be familiar with the following information relating to these risks.

1. **Thromboembolic Disorders and Other Vascular Problems:** An increased risk of thromboembolic and thrombotic disease associated with the use of O.C.s is well established. One British study demonstrated an increased relative risk for fatal venous thromboembolism; two British and three American studies demonstrated an increased relative risk for non-fatal venous thromboembolism. These studies estimate that users of O.C.s are 4 to 11 times more likely than nonusers to develop these diseases without evident cause. **CEREBROVASCULAR DISORDERS.** Two American studies demonstrated an increased relative risk for stroke, which had not been shown in prior British studies. In a collaborative American study of cerebrovascular disorders in women with and without predisposing causes, it was estimated that the relative risk of hemorrhagic stroke was 2.0 times greater in users than nonusers and the relative risk of thrombotic stroke was 4 to 9.5 times greater in users than nonusers. A British long-term, follow-up study reported in 1976 a highly significant association between O.C. use and stroke. Another British long-term, follow-up study had suggested such an association in 1974, but the number of cases was too small to estimate the risk. **MYOCARDIAL INFARCTION.** An increased relative risk of myocardial infarction associated with the use of O.C.s has been reported, confirming a previously suspected association. One study conducted in the United Kingdom found, as expected, that the greater the number of underlying risk factors for coronary artery disease (cigarette smoking, hypertension, hypercholesterolemia, obesity, diabetes, history of preclampsia toxemia) the higher the risk of developing myocardial infarction, regardless of whether the patient was an O.C. user or not. O.C.s, however, were found to be an additional risk factor. In terms of relative risk, it has been estimated that O.C. users who do not smoke (smoking is considered a major predisposing condition to myocardial infarction) are about twice as likely to have a fatal myocardial infarction as nonusers who do not smoke. O.C. users who are also smokers have about a 5-fold increased risk of fatal infarction compared to users who do not smoke, and about a 10- to 12-fold increased risk compared to nonusers who do not smoke. Furthermore, the number of cigarettes smoked is also an important factor. In determining the importance of these relative risks, however, the baseline rates for various age groups must be given serious consideration. (The estimates are based on British vital statistics which show acute myocardial infarction death rates 2- to 3-times less than in the U.S. for women in these age groups; consequently, actual U.S. death rates could be higher.) The importance of other predisposing conditions mentioned above in determining relative and absolute risks has not been quantified; other synergistic actions may exist. **RISK OF DOSE.** In an analysis of data derived from several national adverse reaction reporting systems, British investigators concluded that the risk of thromboembolism including coronary thrombosis is directly related to the dose of estrogen used in O.C.s. Preparations containing 0.1 mg or more of estrogen were associated with a higher risk of thromboembolism than those containing 0.05-0.08 mg of estrogen. Their analysis did suggest, however, that the quantity of estrogen may not be the sole factor involved. This finding has been supported by a study in the United States. Careful epidemiological studies to determine the degree of thromboembolic risk associated with progestogen-only O.C.s have not been performed. Cases of thromboembolic disease have been reported in women using these products, and they should not be presumed to be free of excess risk. **ESTIMATE OF EXCESS MORTALITY FROM CIRCULATORY DISEASE.** A large British prospective study estimated the mortality rate per 100,000 women per year from diseases of the circulatory system for users and nonusers of O.C.s according to age, smoking habits, and duration of use. The overall excess death rate annually from circulatory diseases for O.C. users was estimated to be 20 per 100,000 (ages 15-34—5/100,000; ages 35-44—33/100,000; ages 45-49—140/100,000). The risk is concentrated in older women, in those with a long duration of use, and in cigarette smokers, and may persist after discontinuation of O.C. use. It was not possible, however, to examine the interrelationships of age, smoking, and duration of use, nor to compare the effects of continuous vs. intermittent use. Although the study showed a 10-fold increase in death due to circulatory diseases in users for 5 or more years, all of these deaths occurred in women 35 or older. Until larger numbers of women under 35 with continuous use for 5 or more years are available, it is not possible to assess the magnitude of the relative risk for this younger age group. The available data from a variety of sources have been analyzed to estimate the risk of death associated with various methods of contraception. The estimates of risk of death for each method include the combined risk of the contraceptive method (e.g., thromboembolic and thrombotic disease in the case of O.C.s) plus the risk attributable to pregnancy or abortion in the event of method failure. This latter risk varies with the effectiveness of the contraceptive method. The findings of this analysis are shown in Table 1 below. The study concluded that the mortality associated with all methods of birth control is low and below that of childbirth, with the exception of O.C.s in women over 40 who smoke. (The rates given for pill only/smokers for each age group are for smokers as a class. For "heavy" smokers (more than 15 cigarettes a day), the rates given would be about double; for "light" smokers (less than 15 cigarettes a day), about 50 percent.) The lowest mortality is associated with the condom or diaphragm backed up by stable abortion. The study also concluded that O.C. users who smoke, especially those over the age of 30, have a greater mortality risk than O.C. users who do not smoke.

**TABLE 1.** The annual number of deaths associated with control of fertility and no control per 100,000 nonsterile women by regimen of control and age of women are:

|                                        | 15-19 | 20-24 | 25-29 | 30-34 | 35-39 | 40-44 |
|----------------------------------------|-------|-------|-------|-------|-------|-------|
| No method                              | 5.6   | 6.1   | 7.4   | 13.9  | 20.8  | 22.6  |
| Abortion only                          | 1.2   | 1.6   | 1.8   | 1.7   | 1.9   | 1.2   |
| Pill only—non-smokers                  | 1.3   | 1.4   | 1.4   | 2.2   | 4.5   | 7.1   |
| Pill only—smokers                      | 1.5   | 1.6   | 1.6   | 10.8  | 13.4  | 58.9  |
| IUDs only                              | 0.9   | 1.0   | 1.2   | 1.4   | 2.0   | 1.9   |
| Traditional contraception only         | 1.1   | 1.6   | 2.0   | 3.6   | 5.0   | 4.2   |
| Traditional contraception and abortion | 0.2   | 0.2   | 0.3   | 0.3   | 0.3   | 0.2   |

The risk of thromboembolic and thrombotic disease associated with O.C.s increases with age after approximately age 30 and, for myocardial infarction, is further increased by hypertension, hyperlipidemia, obesity, diabetes, or history of preclampsia toxemia and especially by cigarette smoking. Based on

the data currently available, the following table gives a gross estimate of the risk of death from circulatory disorders associated with the use of O.C.s:

**TABLE 2. Smoking habits and other predisposing conditions—risk associated with use of O.C.s**

|                                       | Age | Below 30 | 30-39 | 40+ |
|---------------------------------------|-----|----------|-------|-----|
| Heavy smokers                         |     | C        | B     | A   |
| Light smokers                         |     | D        | C     | B   |
| Non-smokers                           |     | D        | C,D   | C   |
| (No predisposing conditions)          |     | D        | C,D   | C   |
| Non-smokers                           |     | C        | C,B   | B,A |
| (Other predisposing conditions)       |     | C        | C,B   | B,A |
| A—Use associated with very high risk. |     |          |       |     |
| B—Use associated with high risk.      |     |          |       |     |
| C—Use associated with moderate risk.  |     |          |       |     |
| D—Use associated with low risk.       |     |          |       |     |

The physician and the patient should be alert to the earliest manifestations of thromboembolic and thrombotic disorders (e.g., thrombophlebitis, pulmonary embolism, cerebrovascular insufficiency, coronary occlusion, retinal thrombosis, and mesenteric thrombosis). Should any of these occur or be suspected, the drug should be discontinued immediately. A four- to six-fold increased risk of post-surgery thromboembolic complications has been reported in O.C. users. If feasible, O.C.s should be discontinued at least 4 weeks before surgery of a type associated with an increased risk of thromboembolism or prolonged immobilization. Data also suggest that the presence of varicose veins substantially increases the risk of superficial venous thrombosis of the leg, the risk depending on the severity of the varicosities. **Ocular Lesions:** There have been reports of neuro-ocular lesions such as optic neuritis or retinal thrombosis associated with the use of O.C.s. Discontinue O.C. medication if there is unexplained, sudden or gradual, partial or complete loss of vision; onset of proptosis or diplopia; papilledema; or retinal vascular lesions; and institute appropriate diagnostic and therapeutic measures. **Carcinoma:** Long-term continuous administration of either natural or synthetic estrogen in certain animal species increases the frequency of certain adenomas, benign or malignant, such as those of the breast, cervix, vagina, uterus, ovary, pituitary and liver. Certain synthetic progestogens, none currently contained in O.C.s, have been noted to increase the incidence of mammary nodules, benign and malignant, in dogs. Several retrospective case-control studies have reported an increased relative risk (3.1 to 13.9 times) associating endometrial carcinoma with the prolonged use of estrogens in postmenopausal women who took estrogen replacement medication to relieve menopausal symptoms. One publication reported on the first 30 cases submitted by physicians to a registry of cases of adenocarcinoma of the endometrium in women under 40 on O.C.s. Of the adenocarcinomas found in women without predisposing risk factors for adenocarcinoma of the endometrium (e.g., irregular bleeding at the time O.C.s were first given, polycystic ovaries) nearly all occurred in women who had used a sequential O.C. These products are no longer marketed. No statistical association has been reported suggesting an increased risk of endometrial cancer in users of conventional combination or progestogen-only O.C.s, although individual cases have been reported. Several studies have shown no increased risk of breast cancer to women taking O.C.s or estrogens. In one study, however, while no overall increased risk of breast cancer was noted in women treated with O.C.s, a greater risk was suggested for the subgroups of O.C. users with documented benign breast disease and for long-term (2-4 years) users. One other study indicated an increasing risk of breast cancer in women taking menopausal estrogens, which increased with duration of follow-up. A reduced occurrence of benign breast tumors in users of O.C.s has been well documented. In a prospective study of women with cervical dysplasia, there was an increase in severity and of conversion to cancer *in situ* in O.C. users compared with nonusers. This became statistically significant after 3 to 4 years of use. Nonreversal of dysplasia within the first 6 months of pill use was suggested to be predictive of progression after prolonged exposure. There have been other reports of macroglial hyperplasia of the cervix in users of O.C.s. In summary, there is at present no confirmed evidence from human studies of an increased risk of cancer associated with O.C.s. Close clinical surveillance of all women taking O.C.s is, nevertheless, essential. In all cases of undiagnosed persistent or recurrent abnormal vaginal bleeding, appropriate diagnostic measures should be taken to rule out malignancy. Women with a strong family history of breast cancer or who have breast nodules, fibrocystic disease or abnormal mammograms should be monitored with particular care if they elect to use O.C.s instead of other methods of contraception. **4. Liver Tumors:** Sudden severe abdominal pain or shock may be due to rupture and hemorrhage of a liver tumor. There have been reports associating benign or malignant liver tumors with O.C. use. This has been reported in short-term as well as long-term users of O.C.s. One study reported that use of O.C.s with high hormonal potency and age over 30 years may further increase a woman's risk of hepatocellular adenoma. Two studies relate risk with duration of use, the risk being much greater after 4 or more years of use. Although it is an uncommon lesion, it should be considered in women presenting with an "acute abdomen". The tumor may cause serious or fatal hemorrhage. Patients with liver tumors have demonstrated variable clinical features which may make preoperative diagnosis difficult. Some cases presented because of right upper quadrant masses, while most had signs and symptoms of acute intraperitoneal hemorrhage. Routine radiological and laboratory studies may not be helpful. Liver scans may clearly show a focal defect. Hepatic arteriography may be a useful procedure in diagnosing primary liver neoplasms. **5. Use in or Immediately Preceding Pregnancy, Birth Defects in Offspring, and Malignancy in Female Offspring:** The use of female sex hormones—both estrogenic and progestational agents—during early pregnancy may seriously damage the offspring. It has been shown that females exposed *in utero* to diethylstilbestrol, a nonsteroidal estrogen, have an increased risk of developing in later life a form of vaginal or cervical cancer that is ordinarily extremely rare. This risk has been estimated to be of the order of 1 in 1,000 exposures or less. Although there is no evidence at the present time that O.C.s further enhance the risk of developing this type of malignancy, such patients should be monitored with particular care if they elect to use O.C.s instead of other methods of contraception. Furthermore, a high percentage of women exposed to diethylstilbestrol (from 30 to 90%) have been found to have epithelial changes of the vagina and cervix. Although these changes are histologically benign, it is not known whether this condition is a precursor of vaginal malignancy. Male children so exposed may develop abnormalities of the urogenital tract. Although similar data are not available with the use of other estrogens, it cannot be presumed that they would not induce similar changes. An increased risk of congenital anomalies, including heart defects and limb defects, has been reported following use of sex hormones, including O.C.s, in pregnancy. In one case-control study it was estimated that there was a 4.7-fold increased relative risk of limb-reduction defects in infants exposed *in utero* to sex hormones (O.C.s, hormonal withdrawal tests for pregnancy or attempted treatment for threatened abortion). Some of these exposures were very short and involved only a few days of treatment. The data suggest that the risk of limb-reduction defects in exposed fetuses is somewhat less than 1 in 1,000 live births. In a large prospective study, cardiovascular defects in children born to women who received female hormones, including O.C.s, during early pregnancy occurred at a rate of 18.2 per 1,000 births, compared to 7.8 per 1,000 for children not so exposed *in utero*. These results are statistically significant. In the past, female sex hormones have been used during pregnancy in an attempt to treat threatened or habitual abortion. There is considerable evidence that estrogens are ineffective for these indications, and there is no evidence from well controlled studies that progestogens are effective for these uses. There is some evidence that triploidy and possibly other types of polyploidy are increased among abortuses from women who become pregnant soon after ceasing O.C.s. Embryos with these anomalies are virtually always aborted spontaneously. Whether there is an overall increase in spontaneous abortion of pregnancies conceived soon after stopping O.C.s is unknown. If the patient has not adhered to the prescribed schedule, the possibility of pregnancy should be considered at the time of the first missed period (or after 45 days from the last menstrual period if progestogen-only O.C.s are used) and further use of O.C.s should be withheld until pregnancy has been ruled out. It is recommended that for any patient who has missed two consecutive periods, pregnancy should be ruled out before continuing the contraceptive regimen. If pregnancy is confirmed, the patient should be apprised of the potential risks to the fetus and the advisability of continuing the pregnancy should be discussed in the light of these risks. It is also recommended that women who discontinue O.C.s with the intent of becoming pregnant use an alternate form of contraception for a period of time before attempting to conceive. Many clinicians recommend 3 months, although no precise information is available on which to base this recommendation. The administration of progestogen-only or progestogen-estrogen combinations to induce withdrawal bleeding should not be used as a test of

pregnancy. **6. Gall Bladder Disease:** Studies report an increased risk of gall bladder disease in users of O.C.s or estrogens. In one study, an increased risk appeared after 2 years of use and doubled after 4 or 5 years of use. In another study an increased risk was apparent between 6 and 12 months of use. **7. Carbohydrate and Lipid Metabolic Effects:** A decrease in glucose tolerance has been observed in a significant percentage of patients on O.C.s. For this reason, prediabetic and diabetic patients should be carefully observed while receiving O.C.s. An increase in triglycerides and total phospholipids has been observed in patients receiving O.C.s. The clinical significance of this finding is unknown. **8. Elevated Blood Pressure:** An increase in blood pressure has been reported with O.C. use. In some women, hypertension may occur within a few months of beginning O.C. use. In the first year of use, the prevalence of women with hypertension is low in users and may be no higher than that of a comparable group of nonusers. The prevalence in users increases, however, with longer exposure, and in the fifth year of use is two and a half to three times the reported prevalence in the first year. Age is also strongly correlated with the development of hypertension in O.C. users. Women with a history of elevated blood pressure (hypertension), preexisting renal disease, a history of toxemia or elevated blood pressure during pregnancy, a familial tendency to hypertension or its consequences, or a history of excessive weight gain or fluid retention during the menstrual cycle may be more likely to develop elevation of blood pressure when given O.C.s and, therefore, should be monitored closely. Even though elevated blood pressure may remain within the "normal" range, the clinical implications of elevations should not be ignored and close surveillance is indicated, particularly for women with other risk factors for cardiovascular disease or stroke. High blood pressure may or may not persist after discontinuation of the O.C. **9. Headache:** The onset or exacerbation of migraine or development of headache of a new pattern which is recurrent, persistent, or severe, requires discontinuation of O.C.s and evaluation of the cause. **10. Bleeding Irregularities:** Breakthrough bleeding, spotting, and missed menses are frequent reasons for patients discontinuing O.C.s. In breakthrough bleeding, as in all cases of irregular bleeding from the vagina, nonfunctional causes should be borne in mind. In undiagnosed persistent or recurrent abnormal bleeding from the vagina, adequate diagnostic measures are indicated to rule out pregnancy or malignancy. If pathology has been excluded, time or a change to another formulation may solve the problem. Changing to an O.C. with a higher estrogen content, while potentially useful in minimizing menstrual irregularity, should be done only if necessary since this may increase the risk of thromboembolic disease. Women with a past history of oligomenorrhea or secondary amenorrhea or young women without regular cycles may have a tendency to remain anovulatory or to become amenorrheic after discontinuation of O.C.s. Women with these preexisting problems should be advised of this possibility and encouraged to use other contraceptive methods. Post-use anovulation, possibly prolonged, may also occur in women without previous irregularities. A higher incidence of galactorrhea and of pituitary tumors (e.g., adenomas) has been associated with amenorrhea in former users compared with nonusers. One study reported a 16-fold increased prevalence of pituitary prolactin-secreting tumors among patients with postpill amenorrhea when galactorrhea was present. **11. Infertility:** There is evidence of impairment of fertility in women discontinuing O.C.s in comparison with those discontinuing other methods of contraception. The impairment appears to be independent of the duration of use of the preparations. While the impairment diminishes with time, there is still an appreciable difference in the results in nulliparous women for the O.C. and non-O.C. groups 30 months after discontinuation of birth control. For parous women the difference is no longer apparent 30 months after cessation of contraception. **12. Ectopic Pregnancy:** Ectopic as well as intrauterine pregnancy may occur in contraceptive failures. However, in progestogen-only O.C. failures, the ratio of ectopic to intrauterine pregnancies is higher than in women who are not receiving O.C.s, since the drugs are more effective in preventing intrauterine than ectopic pregnancies. **13. Breast Feeding:** O.C.s given in the postpartum period may interfere with lactation. There may be a decrease in the quantity and quality of the breast milk. Furthermore, a small fraction of the hormonal agents in O.C.s has been identified in the milk of mothers receiving these drugs. The effects, if any, on the breast fed child have not been determined. If feasible, O.C.s should be deferred until the infant has been weaned. **PRECAUTIONS GENERAL:** 1. A complete medical and family history should be taken prior to the initiation of O.C.s. The pretreatment and periodic physical examinations should include special reference to blood pressure, breasts, abdomen and pelvic organs, including Papanicolaou smear and relevant laboratory tests. As a general rule, O.C.s should not be prescribed for longer than 1 year without another physical examination being performed. 2. Under the influence of estrogen-progestogen preparations, preexisting uterine leiomyomata may increase in size. 3. Patients with a history of psychiatric depression should be carefully observed and the drug discontinued if depression recurs to a serious degree. Patients becoming significantly depressed while taking O.C.s should stop the medication and use an alternate method of contraception in an attempt to determine whether the symptom is drug related. 4. O.C.s may cause some degree of fluid retention. They should be prescribed with caution, and only with careful monitoring, in patients with conditions which might be aggravated by fluid retention, such as convulsive disorders, migraine syndrome, asthma or cardiac, hepatic or renal insufficiency. 5. Patients with a past history of jaundice during pregnancy have an increased risk of recurrence of jaundice while receiving O.C. therapy. If jaundice develops in any patient receiving such drugs, the medication should be discontinued. 6. Steroid hormones may be poorly metabolized in patients with impaired liver function and should be administered with caution in such patients. 7. O.C. users may have disturbances in normal tryptophan metabolism which may result in a relative pyridoxine deficiency. The clinical significance of this is unknown. 8. Serum folate levels may be depressed by O.C. therapy. Since the pregnant woman is predisposed to the development of folate deficiency and the incidence of folate deficiency increases with increasing gestation, it is possible that if a woman becomes pregnant shortly after stopping O.C.s, she may have a greater chance of developing folate deficiency and complications attributed to this deficiency. 9. The pathologist should be advised of O.C. therapy when relevant specimens are submitted. 10. Certain endocrine and liver function tests and blood components may be affected by estrogen-containing O.C.s. For example: a. Increased sulfobromophthalen retention. b. Increased prothrombin and factors VII, VIII, IX, and X. c. Decreased antithrombin III. c. Increased norepinephrine-induced platelet aggregability. c. Increased thyroid binding globulin (TBG) leading to increased circulating total thyroid hormone, as measured by protein-bound iodine (PBI), T4 by column, or T4 by radioimmunoassay. Free T3 resin uptake is decreased, reflecting the elevated TBG. Free T4 concentration is unaltered. d. Decreased pregnandiol excretion. e. Reduced response to metyrapone test. **DRUG INTERACTIONS** O.C.s may be rendered less effective by virtue of drug interaction with rifampin, isoniazid, ampicillin, tetracycline, neomycin, penicillin V, chloramphenicol, sulfonamides, nitrofurantoin, barbiturates, phenytoin, primidone, analgesics, tranquilizers, and antimigraine preparations. O.C.s may alter the effectiveness of other types of drugs, such as oral anticoagulants, anticonvulsants, tricyclic antidepressants, antihypertensives (e.g., guanethidine), vitamins and hypoglycemic agents. **CARCINOGENESIS** See Warnings. **PREGNANCY** Pregnancy category X. See Contraindications and Warnings. **NURSING MOTHERS** See Warnings. **ADVERSE REACTIONS** An increased risk of the following serious adverse reactions has been associated with the use of O.C.s (see Warnings): thrombophlebitis, thrombosis, pulmonary embolism, coronary thrombosis, cerebral thrombosis, mesenteric thrombosis, liver tumors, cerebral hemorrhage, hypertension, gall bladder disease, congenital anomalies, neuro-ocular lesions, e.g., retinal thrombosis and optic neuritis. The following adverse reactions have been reported in patients receiving O.C.s and are believed to be drug related: bleeding irregularities (breakthrough bleeding, spotting, missed menses, duration of treatment, amenorrhea after treatment), gastrointestinal symptoms (nausea, vomiting, bloating, abdominal cramps), dysmenorrhea, infertility after discontinuation of treatment, edema, chloasma or melasma which may persist after drug is discontinued, breast changes (tenderness, enlargement, and secretion), intolerance to contact lenses, change in corneal curvature (steepening) change in weight (increase or decrease), change in cervical erosion and cervical secretion, possible diminution in lactation when given immediately postpartum, cholestatic jaundice, migraine, increase in size of uterine leiomyomata, rash (allergic), menorrhagia, reduced tolerance to carbohydrates, vaginal candidiasis, prolactin-secreting pituitary tumors. The following adverse reactions have been reported in users of O.C.s, and the association has been neither confirmed nor refuted: premenstrual-like syndrome, cataracts, changes in libido, chorea, changes in appetite, cystitis-like syndrome, headache, nervousness, dizziness, hirsutism, loss of hair, erythema multiforme, erythema nodosum, hemorrhagic eruption, vaginitis, porphyria, impaired renal function. **INFORMATION FOR THE PATIENT** (See Patient Package Insert)



# American Journal of Obstetrics and Gynecology

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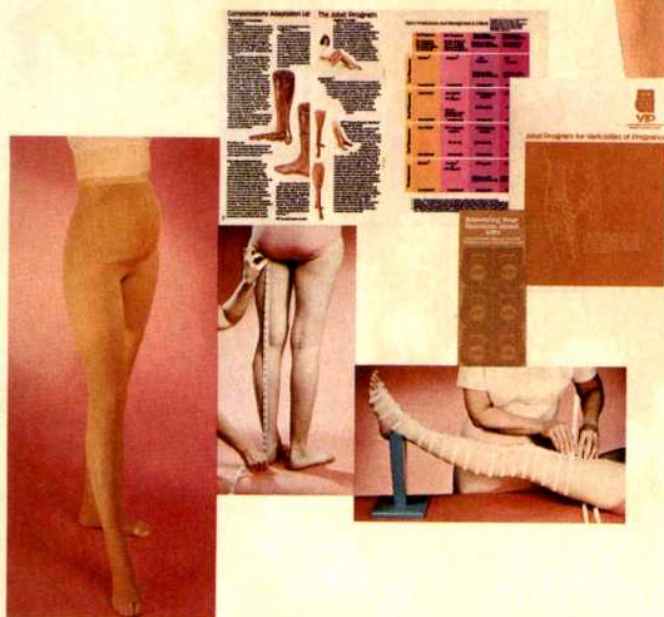
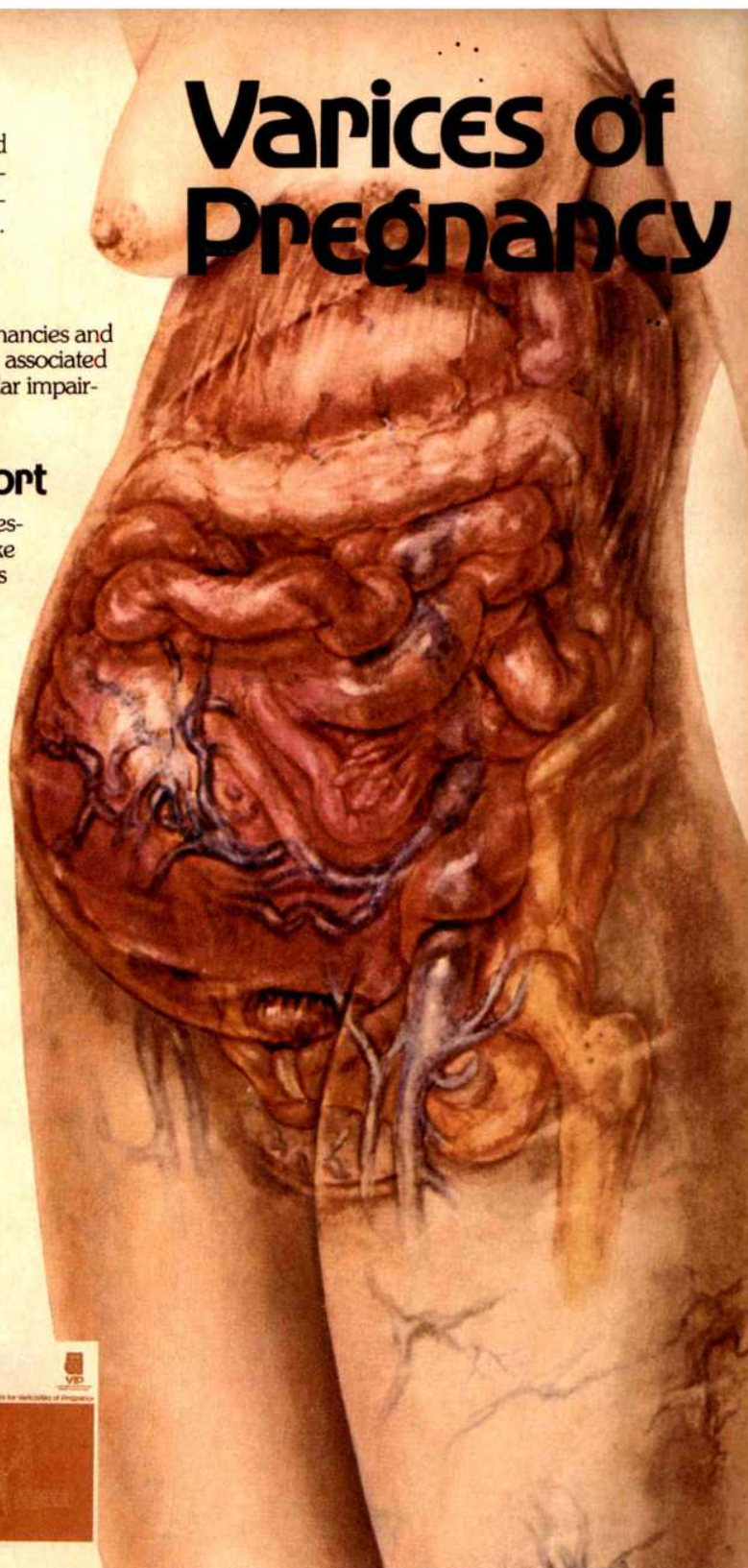
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**Evaluation of elective repeat cesarean section as a standard of care: An application of decision analysis**

KIRKWOOD K. SHY, M.D., M.P.H.

JAMES P. LOGERFO, M.D., M.P.H.

LAURENCE E. KARP, M.D.

*Seattle, Washington*

The rationale for routine repeat cesarean section (RCS) is avoidance of uterine rupture during labor. However, the incidence of uterine rupture following modern cesarean section is low, and the charge for cesarean section is greater than that for a trial of labor (TOL). The technique of decision analysis was used to investigate strategies of elective RCS and TOL with precautionary monitoring. Hypothetical cohorts of 10,000 pregnant women with previous low-transverse cesarean incisions were assumed. Probability data were obtained from the literature. In the TOL cohort, 6,623 patients were delivered vaginally. Uterine rupture occurred in 73 patients. In spite of this, TOL resulted in 37 fewer perinatal deaths and 0.7 fewer maternal deaths than elective RCS. The excessive perinatal mortality in the RCS cohort was related to iatrogenic prematurity that is now avoidable with the lecithin/sphingomyelin test. Direct costs were \$5 million greater in the RCS cohort. We concluded that in contemporary practice mortality rates are essentially equal for both delivery practices. However, substantial cost savings are available with TOL. (AM. J. OBSTET. GYNECOL. 139:123, 1981.)

*From the Robert Wood Johnson Clinical Scholars Program, the Department of Obstetrics and Gynecology, and the Department of Medicine, University of Washington School of Medicine.*

*Presented in part at a meeting of the American Federation of Clinical Research, Washington, D. C., May 7, 1979.*

*The opinions expressed herein are those of the authors and do not necessarily reflect those of the Robert Wood Johnson Foundation.*

*Reprint requests: Dr. Kirkwood K. Shy, Department of Obstetrics and Gynecology, RH-20, University of Washington, Seattle, Washington 98195.*

THE DICTUM "once a Caesarean always a Caesarean" was first put forth by Cragin<sup>1</sup> before the New York State Medical Society in 1916. This concept gained wide support in the United States: in a 1974 Professional Activity Study (PAS) more than 99% of women with previous cesarean sections were delivered by repeat sections in subsequent pregnancies.<sup>2</sup> This standard of care evolved from the considerable body of published data on the frequency of hazardous consequences of uterine rupture after a vertical, classical cesarean incision.<sup>3</sup> However, in contemporary practice, a transverse incision through the lower uterine segment is generally the procedure of choice, and recently this incision has been used for more than 99% of all cesarean sections.<sup>2</sup>

Transverse scars rupture less often than classical scars; in addition, when uterine rupture does occur after a transverse incision, the associated perinatal and maternal mortality is substantially less.<sup>3, 4</sup> Because of the increased relative safety of the transverse scar, repeat cesarean section (RCS) as the standard of care has been the subject of debate among obstetricians. In fact, in some British centers, the majority of women have had a trial of labor (TOL) after a previous cesarean section.

Since randomized clinical trials are unlikely to be forthcoming, we used the technique of decision analysis to consider the optimal planned route of delivery after a previous cesarean section. Decision analysis offers a mathematical framework to study the logic of medical and surgical decisions that otherwise might be left to chance or dogma. Since mortality rates and dollar costs are the measures of greatest interest to clinicians and planners, these outcomes were emphasized in our analysis. Sufficient data on perinatal and maternal morbidity after RCS and TOL are not available.

## Methods

There are four fundamental components of decision analysis<sup>5, 6</sup>: The first step is the construction of a *decision tree*. This is a flow diagram that specifies alternative courses of action and possible outcomes of these actions. The second component is the estimation of *probabilities*. A branch point in the decision tree where outcome is determined by chance is called a *chance node*. Each chance node has an associated probability estimated from the medical literature. The third step is the assignment of *relative values*. Relative values with the same unit are assigned to all outcomes. The values are expressed in units such as dollar costs or deaths. Fourth, a score for each decision alternative is calculated by multiplying the relative value for each outcome times the probability of its occurrence and sum-

ming relative values across all the branches of each decision alternative.

### Construction of the decision tree (Figs. 1 and 2).

The alternative for the pregnant woman with a previous low-transverse cesarean section is a planned TOL or a planned RCS. Although a RCS may be performed after the onset of labor or after premature rupture of the membranes, in the United States most RCSs are scheduled for an elective date prior to the onset of labor. Consequently, in our analysis, only elective RCS was included in the decision tree; however, the strategy of permitting the onset of labor prior to RCS is considered in the discussion.

Even if a RCS is planned, spontaneous uterine rupture can occur prior to the onset of labor (chance node A). When rupture occurs, emergency abdominal delivery is necessary. (Uterine rupture was defined as a uterine defect that was either symptomatic with pain or bleeding or asymptomatic but requiring therapy. This definition excluded the asymptomatic uterine "window" occasionally found at RCS, since the uterine window has no associated mortality or morbidity.) If no rupture occurs, the patient can begin labor spontaneously and have an "emergency" cesarean section, or the RCS can be scheduled (chance node B). Chance node C followed elective cesarean section. It distinguished mature from iatrogenically premature infants.

If a TOL is planned, prelabor uterine rupture can also occur spontaneously (chance node 1). Chance node 2 specified uterine rupture during labor. RCS is indicated after the onset of labor for cephalopelvic disproportion (CPD) or other complications (chance node 3). Perinatal mortality is a possibility at each of the branches of the decision tree. The risk of perinatal mortality was expressed with a final series of chance nodes: chance nodes D to G for planned RCS and chance nodes 4 to 7 for planned TOL.

**Estimation of probabilities (Table I).** The probabilities were estimated from review of the English language medical literature since 1950. Most of the studies were American or British. (A complete list of the references that were used to generate the probabilities in Table I is available from the authors on request.)

For the RCS cohort, data were available to estimate individually the probabilities of prematurity from two causes: (1) iatrogenic prematurity associated with elective RCS prior to labor (chance node C) and (2) noniatrogenic prematurity associated with spontaneous labor prior to the scheduled elective cesarean section date (chance node B). For the RCS cohort, data were also available to estimate individually the probability of perinatal mortality from each of the two sources of prematurity. For planned TOL, the literature did not



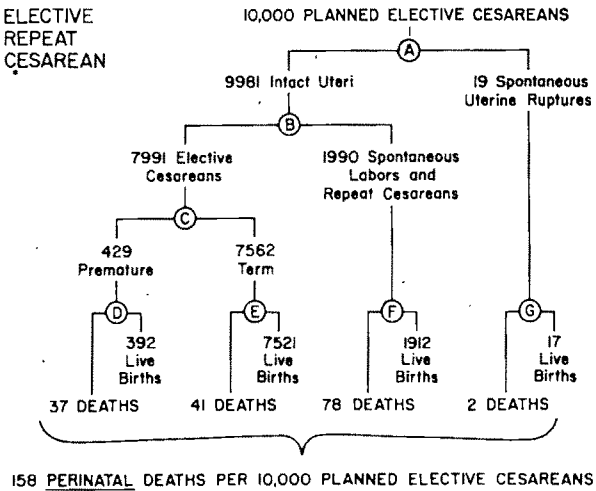


Fig. 1. Perinatal mortality decision tree for planned elective cesarean section. Circles indicate chance nodes.

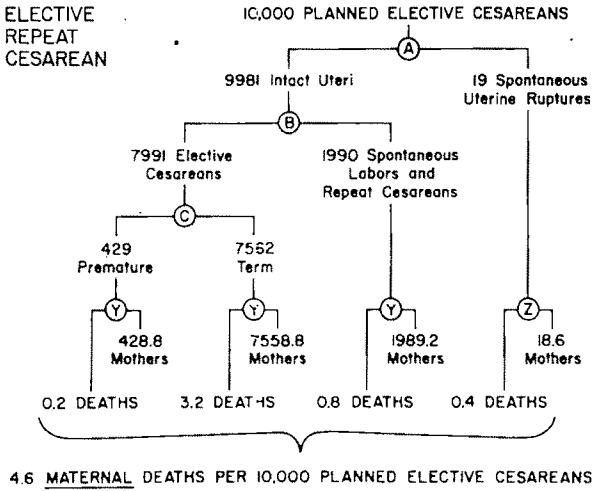


Fig. 3. Maternal mortality decision tree for planned elective cesarean section. Circles indicate chance nodes.

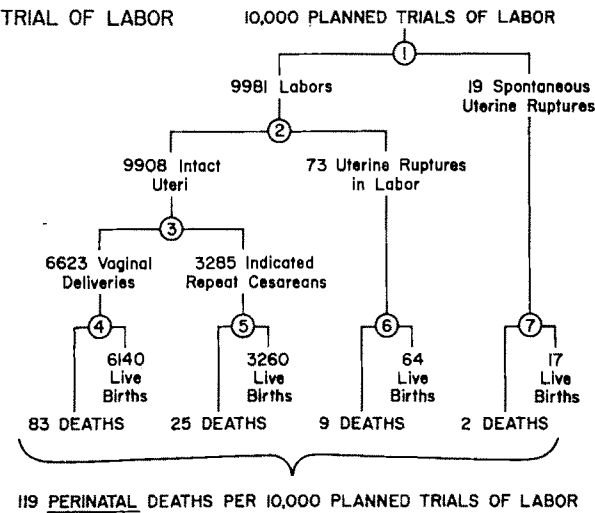


Fig. 2. Perinatal mortality decision tree for planned TOL. Circles indicate chance nodes.

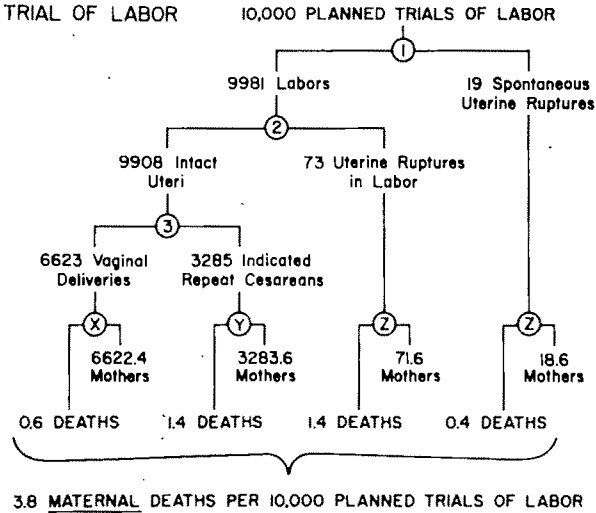


Fig. 4. Maternal mortality decision tree for TOL. Circles indicate chance nodes.

specify whether the associated perinatal mortality resulted from a premature birth. Therefore, at chance nodes 4 through 7, a fraction of the estimated perinatal mortality was probably related to prematurity.

The indication for the primary cesarean section influenced the outcome of a TOL during subsequent pregnancies. If the indication for the primary cesarean section was recurrent, such as CPD, then the chance of vaginal delivery was less than if the indication for the primary cesarean section was nonrecurrent (e.g., breech presentation, placenta previa, etc.).<sup>7, 8</sup> The indication for the primary cesarean section also affected the incidence of uterine rupture.<sup>7, 8</sup> The references at chance nodes 2 and 3 were restricted to papers from

clinics where the indication for the primary cesarean section did not influence patient selection for TOL. This avoided an undue representation of data from patients with nonrecurrent indications for primary cesarean section.

In Table I cases were summed from the individual articles to give the total number of patients and the total number affected. (Perinatal mortality statistics were corrected wherever possible for stillbirth prior to labor, major congenital malformations, erythroblastosis fetalis, and birth weight less than 1,000 gm.) An exception to this policy occurred. Only one article<sup>9</sup> was available to estimate the probability of perinatal mortality after spontaneous uterine rupture (chance node

Table I. Source and magnitude of perinatal p-obability estimate

| Chance node                                      | No. of litera-<br>ture references | Total No.<br>affected* | Total No.<br>of patients* | Chance node<br>probability† |
|--------------------------------------------------|-----------------------------------|------------------------|---------------------------|-----------------------------|
| <i>Planned TOL:</i>                              |                                   |                        |                           |                             |
| 1. Spontaneous uterine rupture                   | 3                                 | 5                      | 2,627                     | 0.0019                      |
| 2. Uterine rupture in labor                      | 5                                 | 35                     | 4,784                     | 0.0073                      |
| 3. Vaginal delivery                              | 5                                 | 754                    | 1,128                     | 0.6684                      |
| 4. Mortality with vaginal delivery               | 7                                 | 12                     | 949                       | 0.0126                      |
| 5. Mortality with indicated RCS                  | 5                                 | 3                      | 390                       | 0.0077                      |
| 6. and 7. Mortality with uterine rupture         | 10                                | 14                     | 117                       | 0.1197                      |
| <i>Planned Elective cesarean section:</i>        |                                   |                        |                           |                             |
| A. Spontaneous uterine rupture                   | 3                                 | 5                      | 2,627                     | 0.0019                      |
| B. Spontaneous labor and repeat cesarean section | 2                                 | 131                    | 657                       | 0.1994                      |
| C. Iatrogenic prematurity                        | 8                                 | 315                    | 5,869                     | 0.0537                      |
| D. Mortality with iatrogenic prematurity         | 7                                 | 26                     | 305                       | 0.0852                      |
| E. Mortality with elective mature delivery       | 4                                 | 22                     | 4,088                     | 0.0054                      |
| F. Mortality with spontaneous labor              | 1                                 | 4                      | 102                       | 0.0392                      |
| G. Mortality with spontaneous uterine rupture    | 1                                 | 0                      | 29                        | 0.1197‡                     |

Perinatal death rates were corrected for stillbirth prior to labor, birth weight less than 1,000 gm, erythroblastosis, and malformations incompatible with life.

\*Cases were summed from each of the individual papers to give the total number affected and the total number of patients.

†P =  $\frac{\text{No. affected}}{\text{No. cases}}$

‡Since it is unlikely that the probability of perinatal death after spontaneous uterine rupture is zero, the same perinatal death rate, 0.1197, was used for chance nodes 6, 7, and G.

Table II. Unit costs used in the decision analysis

| Service                                    | Direct cost |           |
|--------------------------------------------|-------------|-----------|
|                                            | Vaginal*    | Cesarean* |
| <i>Delivery:</i>                           |             |           |
| Hospital                                   | \$1,000     | \$1,475   |
| Physician                                  | \$ 400      | \$ 600    |
| Anesthesiologist                           | \$ 155      | \$ 195    |
| Total                                      | \$1,555     | \$2,270   |
| Neonatal intensive care unit <sup>14</sup> | \$2,700     |           |

\*These costs were estimated by R. D. Allen, M.D., Director of King County Medical Blue Shield, Seattle, Washington.

G). Since no perinatal deaths occurred in this series, this estimated risk of perinatal mortality would be zero. However, it is unlikely that a spontaneous, often unattended uterine rupture is associated with a lesser perinatal mortality rate than an attended rupture during a TOL. Thus, we used the same perinatal mortality rate (119.6 perinatal deaths per 1,000 uterine ruptures) in both circumstances.

In the maternal mortality analysis, the probabilities for maternal mortality were substituted for probabilities of perinatal mortality at chance nodes D to G and 4 to 7. Maternal mortality data were obtained from two large PAS studies. The first, the PAS Cesarean Section Study, reported 16 maternal deaths among 37,769 patients with repeat cesarean deliveries,<sup>2</sup> or a

rate of 4.2 maternal deaths per 10,000 RCSs. (The case fatality rate was 8.0 maternal deaths per 10,000 cesarean sections when all cesarean sections were considered.) The second was the 1977 PAS Hospital Mortality Study that reported 139 deaths among 1,582,401 pregnancies when the diagnosis explaining admission was normal delivery.<sup>10</sup> Hence, the maternal mortality rate was 0.88 per 10,000 vaginal deliveries.

Since on thorough literature review we found no instance of maternal death in association with rupture of a low transverse cesarean section scar, the probability of this event was approximated statistically with probability theory.<sup>11</sup> Dewhurst,<sup>3</sup> O'Driscoll,<sup>4</sup> Eames,<sup>12</sup> and Schrimsky and Benson<sup>13</sup> observed no maternal deaths in a total of 115 lower-segment ruptures. For the fraction 0/115, we calculated the 90% upper confidence limit to give a probability estimate of 198 maternal deaths per 10,000 ruptures of transverse lower-segment scars. This probability estimate is a conservative upper bound.

**Assignment of relative values and unit costs (Table II).** Two relative value units were used. The first, which is of greatest interest to the patient and physician, was mortality. At a second stage, dollar values were assigned.

Direct dollar charges were assigned as the costs of hospital, physician, anesthesia, and neonatal intensive care unit (NICU) services. Because charges may not



Table III. Sensitivity analysis: Calculated threshold probabilities\*

| Chance node†                                     | Probability estimate | Threshold probability | 90 % Confidence interval        |
|--------------------------------------------------|----------------------|-----------------------|---------------------------------|
| I. Perinatal mortality                           |                      |                       |                                 |
| 2. Uterine rupture in labor                      | 0.0073               | 0.0407                | 0.0057, 0.0088                  |
| 4. Mortality with vaginal delivery               | 0.0126               | 0.0184                | 0.0080, 0.0172                  |
| 5. Mortality with indicated cesarean section     | 0.0077               | 0.0195                | 0.0020, 0.0134                  |
| 6. Mortality with uterine rupture in labor       | 0.1197               | 0.6667                | 0.0813, 0.1581                  |
| B. Spontaneous labor and repeat cesarean section | 0.1994               | 0.0668                | 0.1794, 0.2194                  |
| F. Perinatal death after spontaneous labor‡      | 0.0392               | 0.0204                | 0.0146, 0.0638                  |
| II. Maternal mortality                           |                      |                       |                                 |
| 2. Uterine rupture in labor                      | 0.0073               | 0.0302                | 0.0057, 0.0088                  |
| 3. Vaginal delivery                              | 0.6684               | 0.4467                | 0.6505, 0.6863                  |
| X. Mortality with vaginal delivery               | 0.00009              | 0.00021               | (7.85, 9.75) × 10 <sup>-5</sup> |
| Y. Mortality with repeat cesarean section‡       | 0.00042              | 0.00030               | (2.85, 5.55) × 10 <sup>-5</sup> |
| Z. Mortality with uterine rupture                | 0.1980               | 0.0301                | (99.3, 1980) × 10 <sup>-5</sup> |

\*The threshold probability is the probability at which the number of perinatal or maternal deaths is equal with that of either a TOL or a planned elective repeat cesarean section.

†No probability can be chosen at chance nodes 1, 7, A, C, D, E, or G that will alter the decision for TOL.

‡The threshold probability at these chance nodes lies within the 90% confidence interval for the estimated chance node probability. Thus, these nodes are *sensitive* (see text for details).

reflect marginal costs, charges for health care do not always reflect the true cost of a product or service. In spite of this and other limitations, charges have intuitive and practical appeal as a proxy for direct dollar costs, and charges were used to approximate direct costs in our analysis.

Results

**Expected perinatal mortality.** The decision tree in Figs. 1 and 2 displays the perinatal outcomes, according to delivery plan, for 10,000 women with previous low transverse cesarean sections. The predicted numbers of perinatal mortalities were calculated with the use of the probabilities from Table I. A planned TOL had a lower expected number of perinatal deaths (119) as compared with the expected number of perinatal deaths for planned RCS (158).

Uterine rupture was rare regardless of the delivery plan. Nineteen ruptures occurred per 10,000 pregnancies with elective RCS, and 92 ruptures occurred with TOL. With planned TOL, only 9.2% (11/119) of the associated perinatal mortality was related to uterine rupture. The rate of iatrogenic prematurity for planned RCS was 5.4% and an 8.5% perinatal mortality rate was associated with these premature deliveries. Elective RCS and iatrogenic prematurity resulted in 3.1 times as many perinatal deaths as planned TOL and uterine rupture. In this analysis there were approximately 39 excess perinatal deaths per 10,000 pregnancies with the plan of elective RCS.

After TOL, approximately 67% of all patients with a cesarean section scar were delivered vaginally. If only

Table IV. Estimated dollar costs of delivery (millions) after previous cesarean section for cohorts of 10,000 pregnancies\*

|                               | Direct costs<br>(millions of dollars) |                           |
|-------------------------------|---------------------------------------|---------------------------|
|                               | TOL                                   | Elective cesarean section |
| Vaginal delivery              | \$10.3                                | —                         |
| Cesarean section              | \$ 7.7                                | \$22.7                    |
| Neonatal intensive care unit† | \$ 0.96                               | \$ 1.3                    |
| Total direct costs            | \$19.0                                | \$24.0                    |

\*Dollar costs for each delivery plan were calculated as the product of the appropriate number of cases from the decision tree (Figs. 1 and 2) and the unit costs from Table II.

†Assumes three neonatal intensive care unit admissions per perinatal death.

patients with a nonrecurrent indication for the primary cesarean section had been considered, the rate of vaginal delivery would have increased to between 70% and 90%.<sup>7, 8</sup>

**Expected maternal death.** Expected maternal deaths were calculated after the appropriate maternal mortality probabilities were substituted into the decision tree. With RCS, 4.6 maternal deaths were calculated versus 3.8 maternal deaths for a planned TOL (Figs. 3 and 4). Thus, in our analysis, the relative risk of maternal mortality with RCS was 1.2 times that of TOL. For the hypothetical cohort of 10,000 parturients with the delivery plan of elective RCS, an excess 0.7 maternal death occurred. Because a statistical upper confidence limit was chosen to estimate the probability of maternal

death with uterine rupture, 0.7 may underestimate the true difference in maternal mortality risk between plans.

**Sensitivity analysis (Table III).** The process of testing whether alterations in chance node probabilities influence a given decision is known as sensitivity analysis.<sup>5</sup> A threshold exists for many probabilities; if this threshold probability is exceeded, then the decision (in this case TOL) would be changed. When reasonable probability variations exceed the threshold (and, therefore, change the decision), a decision is said to be sensitive. If no reasonable probability variation can alter the decision, the decision is insensitive. We calculated 90% (one-tailed) confidence limits for the estimated probabilities at each chance node. If the threshold probability was outside the confidence limits, we considered that chance node to be insensitive.

The analyses of perinatal and maternal mortality were each sensitive at a single chance node. A 48% decrease in our estimated risk of perinatal death after spontaneous labor and RCS (chance node F) would alter the decision in the perinatal analysis, and a 29% decrease in our estimated risk of maternal mortality after RCS (chance node Y) would alter our decision in the maternal mortality analysis.

**Expected dollar costs (Table IV).** The direct costs for a planned RCS exceeded the direct costs of a planned TOL by \$5 million. Most of the cost difference was related to actual delivery costs and averaged \$500 per patient. The magnitude of this cost difference rests on the assumption of equal obstetric charges for vaginal delivery, regardless of previous cesarean section.

### Comment

Our results indicate that a TOL is not associated with a substantially greater risk of death for either the infant or the mother. This is true primarily because uterine rupture contributes relatively little to the overall incidence of perinatal and maternal mortality for patients undergoing a TOL and because the utilization of routine elective cesarean section may be associated with other risks of mortality that counterbalance the risks of uterine rupture. In addition, a TOL offers substantial dollar savings in direct costs.

Before accepting these conclusions, the major limitations of the study should be reviewed. Our mortality results were sensitive to decreases in the probability of perinatal death after spontaneous labor and RCS (chance node F) and the probability of maternal death associated with RCS (chance node Y). To improve confidence in the choice of either route of delivery after previous cesarean section, more data are needed at these chance nodes. Simultaneous changes in the

probabilities at more than one chance node, such as a 300% increase in the rate of uterine rupture in labor (chance node 2) and a 60% decrease in the mortality rate associated with iatrogenic prematurity (chance node D), also would have altered our results. However, simultaneous probability changes were not systematically investigated.

The overall difference in the rates of perinatal mortality between planned TOL and planned elective RCS occurred almost entirely because of the problem of iatrogenic prematurity. However, strategies for elective cesarean delivery are now available to avoid iatrogenic prematurity and the respiratory distress syndrome.<sup>15</sup> Measurement of the lecithin/sphingomyelin (L/S) ratio in amniotic fluid obtained by transabdominal amniocentesis can accurately assess pulmonary maturation<sup>16</sup> and thereby allow elective delivery to be properly timed. Unfortunately, the L/S ratio remains underutilized in some practices.<sup>14</sup> In our analysis, elective RCS was related to iatrogenic prematurity and 37 perinatal deaths. If these deaths had been averted with L/S ratio determinations, perinatal mortality rates between plans would have been almost identical.

The charge for third-trimester amniocentesis and L/S ratio determination at the University of Washington Hospital is \$150, including laboratory, professional, institutional, and ultrasonographic fees. By conservative estimate, this test would have generated additional charges of \$1.2 million for the 7,991 elective RCSs.

A second strategy to eliminate iatrogenic prematurity is to await the onset of labor prior to RCS. Since nine deaths were attributable to uterine rupture in the TOL cohort, nine is the maximum number of perinatal deaths that might be exchanged for a considerable reduction in the 37 perinatal deaths associated with iatrogenic prematurity. However, adoption of this strategy requires consideration of the major secondary risks to the mother, namely, uterine rupture and aspiration pneumonia. Unfortunately, quantification of the maternal risk of this strategy was precluded by a lack of data on the differential rate of aspiration of stomach contents in patients who enter labor prior to RCS.

This report cannot be generalized to the management of all pregnant women with a history of cesarean section. In the studies that advocated a routine TOL, precautionary monitoring was utilized, and this is not always possible in the community practice of obstetrics. Precautions included constant obstetric attendance during labor, ready availability of blood products, and proximity to an operating theater and anesthesia. Further, if the probability of vaginal delivery with a TOL is estimated to be very low (as in many cases of pelvic contractures), then many patients will find a



TOL unacceptable. Physicians should also be reluctant to undertake a TOL when the probability of vaginal delivery is low. From our analysis we estimated that if the probability of a vaginal delivery is less than 0.4467, the risk of maternal mortality is greater with TOL than with RCS (see Table III).

Our study suggests additional data and investigations that are important to clarify the use of TOL after previous cesarean section. Case-control studies on the risk factors for uterine rupture would identify subgroups of patients who are inappropriate for a TOL. Patients with recurrent indications for cesarean section have already been suggested to be at high risk for uterine rupture, but this factor needs to be refined. Patients with two or more previous cesarean sections are another subgroup of interest. Indicated RCS after a failed TOL may be associated with increased risks of maternal operative morbidity. Consequently, this morbidity needs to be better defined. Last, the issue of patient satisfaction is fundamental to the choice of the appropriate route of delivery after previous cesarean section.

Cost issues aside, what proportion of women prefer a TOL in the hopes of a vaginal delivery? What are the psychic costs associated with a failed TOL?

In summary, reasonable outcome comparisons between elective RCS and TOL can be made when available data are examined with decision analysis. To individualize the planned route of delivery for a patient, the practicing obstetrician must utilize these same data and also account for differences in antecedent medical risk and indirect and intangible costs. This is desirable. However, in the general case, we have reached the same conclusion as many of our British colleagues. When precautionary monitoring is utilized, a TOL provides substantial dollar cost savings with no greater mortality risk than the current American standard of RCS.

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# GYNECOLOGY

## Sexually transmitted conditions among women college students

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We studied 500 unselected young women who consulted a gynecologist in a student health service. Most participants were symptom-free and had normal physical examinations. Few sexually transmitted infections were encountered. *Neisseria gonorrhoeae* was recovered from two and *Trichomonas vaginalis* was obtained from 14 of the 500 women. *Chlamydia trachomatis* was recovered from 20 (4.6%) of 439 participants. Genital warts, genital herpes, and molluscum contagiosum, respectively, were noted in seven, four, and one of the 500 participants. There were no cases of syphilis, scabies, or pediculosis pubis. *Mycoplasma hominis* and *Ureaplasma urealyticum*, respectively, were recovered from 17.6% and 56.8% of the subjects. Prevalent vaginal bacteria included lactobacilli, streptococci, *Staphylococcus epidermidis*, and diphtheroids. *Gardnerella vaginalis* was isolated from the vaginal specimens of about one third of the participants. (AM. J. OBSTET. GYNECOL. 139:130, 1981.)

THE OBJECTIVE of this study was to determine the prevalence of sexually transmitted conditions in women. In order to avoid the biases inherent in studying

hospitalized patients or patients attending public clinics, we chose to study a group of relatively healthy college students.

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This report describes the clinical characteristics of 500 women college students, as well as the results of the examination of genital specimens from these women for mycoplasmas, trichomonads, chlamydiae, fungi, and selected aerobic and facultatively anaerobic bacteria. The size of the study population precluded a detailed examination of strictly anaerobic organisms. Such an analysis has been done on a different group of women from the same population.<sup>1</sup> In addition, detailed reports have been prepared that describe the clinical and epidemiologic characteristics of colonization with *Gardnerella vaginalis*,<sup>2</sup> group B *Streptococcus*,<sup>3</sup> and *Chlamydia trachomatis*<sup>4, 5</sup> in this group of subjects.

### Material and methods

Women presenting for any reason to the only gynecologist in the Health Service of a large university were asked to participate in the study. Written informed consent was obtained from those who agreed to participate after a detailed description of the methods that would be used and of the measures that would be used to ensure confidentiality. Each participant completed a questionnaire which contained basic demographic questions as well as questions on sexual development, sexual experience, and gynecologic symptoms. During the physical examination, the gynecologist specifically recorded the presence or absence of a number of abnormal physical findings. Four vaginal cultures were obtained from each participant by rubbing sterile cotton-tipped applicators against the vaginal wall. Two endocervical cultures were also obtained with the use of sterile cotton-tipped applicators.

The first vaginal swab culture was inoculated immediately onto fresh sheep blood-beef heart infusion agar, cross-streaked with a sterile wire loop, incubated in a candle jar for 48 hours at 36.5° C, and examined for aerobic and facultative bacteria with the use of standard methods.

The second vaginal swab culture was broken into a sterile glass vial containing 2.0 ml of mycoplasma transport broth which was kept on wet ice for 2 to 6 hours before being frozen at -80° C. After thawing, the mycoplasma transport broth was examined for *Mycoplasma hominis* and *Ureaplasma urealyticum* (T mycoplasmas) by means of previously described methods.<sup>6</sup>

The third vaginal swab culture was broken into a sterile glass vial containing Trichosel broth (Baltimore Biological Laboratory) which was incubated at 36.5° C. The Trichosel broth was examined every 1 to 2 days for motile *Trichomonas vaginalis*.

The fourth vaginal culture was inoculated into modified Todd-Hewitt broth, incubated at 36.5° C overnight, and frozen at -20° C. After thawing, an aliquot

was inoculated onto blood agar and examined for group B *Streptococcus*.<sup>3</sup>

The first endocervical culture was inoculated onto fresh sheep blood-beef heart infusion agar to which vancomycin, colistin, and nystatin (VCN inhibitor, Baltimore Biological Laboratory) had been added. Previous studies have shown this fresh medium to be equivalent to commercial Thayer-Martin agar for the isolation of *Neisseria gonorrhoeae* from endocervical cultures.<sup>7</sup> Colonies of typical morphology which gave a positive oxidase reaction and which contained gram-negative cocci were presumptively identified as *N. gonorrhoeae*.

The second endocervical specimen was placed into 0.5 ml of sucrose-phosphate solution containing streptomycin (50 µg/ml) nystatin (25 µg/ml), and gentamicin (10 µg/ml). These specimens were kept on wet ice for 2 to 6 hours, then frozen and stored at -80° C. After thawing at 37° C, each specimen for culture was homogenized with a syringe and needle and examined for *C. trachomatis* by the micro-cell culture method with the use of irradiated baby hamster kidney (BHK-21) cells.<sup>4, 5</sup>

To ensure personal anonymity, the questionnaires, the forms on which the physical findings had been recorded, and the cultures were identified only by a code number. The consent form, the only document which contained the patient's name, was filed with her University Health Service medical record. The investigators, other than the gynecologist, did not have access to these records. Similarly, the gynecologist did not have access to the questionnaires.

### Results

Five hundred women were enrolled in the study, 488 of whom completed the confidential questionnaires. Most of the participants were visiting the health service for contraceptive advice and/or for annual cervical cytologic examinations. Clinical observations and cultures for genital mycoplasmas, *N. gonorrhoeae*, and *T. vaginalis* were obtained from all 500 participants. Cultures from 493 individuals were examined for group B *Streptococcus*, vaginal cultures from 466 participants were examined for other vaginal bacteria and fungi, and cervical specimens from 439 young women were examined for *C. trachomatis*.

The 488 participants who completed the questionnaire had a mean age of 20.6 years (range 16 to 35 years). Four hundred thirty-six women were white, 45 were black, and seven were of other races. All except 74 were undergraduate students. Only 10 (2.1%) of the participants were seen during the first 6 days of their menstrual cycle, presumably because women tend to

**Table I.** Selected aerobic and facultatively anaerobic bacteria and fungi isolated from vaginal cultures of 466 women

| Organism                                              | No. isolated | % isolated |
|-------------------------------------------------------|--------------|------------|
| Gram-positive rods:                                   |              |            |
| <i>Gardnerella vaginalis</i>                          | 150          | 32.2       |
| Gram-negative rods:                                   |              |            |
| <i>Escherichia coli</i>                               | 55           | 11.8       |
| <i>Proteus mirabilis</i>                              | 8            | 1.7        |
| <i>Klebsiella</i> species                             | 6            | 1.2        |
| <i>Haemophilus influenzae</i>                         | 5            | 1.0        |
| <i>Acinetobacter calcoaceticus</i> var. <i>lwoffi</i> | 2            | 0.4        |
| <i>Enterobacter</i> species                           | 2            | 0.4        |
| <i>Haemophilus parahaemolyticus</i>                   | 1            | 0.2        |
| <i>Proteus morganii</i>                               | 1            | 0.2        |
| Gram-positive cocci:                                  |              |            |
| Group B <i>Streptococcus</i>                          | 44           | 9.3        |
| Group D <i>Streptococcus</i>                          | 21           | 4.5        |
| <i>Staphylococcus aureus</i>                          | 12           | 2.6        |
| Gram-negative cocci:                                  |              |            |
| <i>Neisseria gonorrhoeae</i>                          | 2            | 0.4        |
| Fungi:                                                |              |            |
| <i>Candida albicans</i>                               | 61           | 13.1       |
| <i>Torulopsis glabrata</i>                            | 9            | 1.9        |
| <i>Candida tropicalis</i>                             | 1            | 0.2        |
| Not speciated                                         | 5            | 1.0        |

avoid routine vaginal examinations while they are menstruating. Their mean age at menarche was 12.6 years (median 13 years, range 9 to 17 years). Most (468) used tampons to absorb their menstrual flow.

Forty-eight participants had been pregnant but only two were known to be pregnant at the time they were studied. Eleven participants had a history of previous gonococcal infection; one woman had had syphilis. During the week before participation in the study, 40 (8.2%) of the women had experienced dysuria, 76 (15.6%) reported lower abdominal pain, and 99 (20.3%) had noted an unusual vaginal discharge.

Of the 486 women who answered the questions about sexual experience, 427 (87.9%) were sexually experienced. Two hundred thirty-six of the sexually experienced women used oral contraceptives, 90 used diaphragms, 25 relied on condoms, 18 had intrauterine contraceptive devices, and 32 used other methods or combinations of methods of contraception. Twenty sexually experienced women used rhythm and/or withdrawal. Six sexually experienced women employed no contraceptive measures. Most (350, 71.8%) of the participants did not smoke cigarettes. All but 29 had never been married. Their mothers and fathers, respectively, had had an average of 15.2 and 16.7 years of formal education.

The examining gynecologist diagnosed genital warts in seven, genital herpes in four, and molluscum contagiosum in one of the 500 participants. None of the

women was found to have syphilis, scabies, or pediculosis pubis. An abnormal vaginal discharge was noted on examination of 86 (17.2%) of the women.

*N. gonorrhoeae* was recovered from two (0.4%), *T. vaginalis* from 14 (2.8%), *M. hominis* from 88 (17.6%) and *U. urealyticum* (T strain mycoplasmas) from 284 (56.8%) of the 500 participants. *C. trachomatis* was recovered from 20 (4.6%) of the 439 women whose genital specimens were examined for this organism. Streptococci, *Staphylococcus epidermidis*, diphtheroids, and lactobacilli were isolated frequently from vaginal specimens. Each of these organisms was recovered from at least 25% of the participants.

Some of the other aerobic and facultatively anaerobic organisms isolated from the 466 participants whose vaginal cultures were examined are listed in Table I. Enteric organisms were encountered infrequently. *Escherichia coli* was recovered from 55 (11.8%) and group D streptococci from 21 (4.5%) of the 466 women. Other enteric organisms such as *Klebsiella* species and *Proteus mirabilis* were isolated from less than 2% of participants. As indicated above, *N. gonorrhoeae* was isolated from only two of the participants. There were no isolations of *Neisseria meningitidis* or of other species of *Neisseria*.

Group B streptococci were isolated from 44 (9.4%) of the 466 specimens which were inoculated onto blood agar and from 85 (17.2%) of the 493 specimens which were subcultured onto blood agar after overnight incubation in Todd-Hewitt broth. These data which confirm the superiority of preincubation in selective broth before inoculation over direct inoculation of blood agar for the isolation of group B streptococci have been reported in detail elsewhere.<sup>3, 8</sup>

*Candida albicans*, the most prevalent fungal species, was isolated from 61 (13.1%) of participants. *Torulopsis glabrata* was recovered from vaginal specimens from nine women.

**Comment**

In order to determine the prevalence of sexually transmitted conditions among relatively healthy women, we studied a group of college students who presented to the gynecologist in a student health service. Most of the women were symptom-free and were being seen for routine physical examinations. Most of these examinations were normal. The classical sexually transmitted diseases were encountered infrequently. There were only two patients with gonococcal infection. It is of interest that chlamydial infections were 10 times as prevalent (20 women). There were 14 women who had trichomoniasis, seven with genital warts, and four with genital herpetic infection. No cases of syphi-



lis, scabies, or pediculosis pubis were seen. These data indicate that this group of students, although almost 90% of them were sexually experienced, had a low prevalence of sexually transmitted conditions.

*Ureaplasma urealyticum* was recovered from 56.8% and *Mycoplasma hominis* from 12.6% of the participants. These data are in accord with an earlier study in which we showed these organisms to be common components of the vaginal microbial flora of normal sexually experienced women.<sup>9</sup>

Our data are in general agreement with those presented in other studies of vaginal microbial flora.<sup>10, 11</sup> Commensals such as lactobacilli, streptococci, *S. epidermidis*, and diphtheroids were the organisms which were isolated most often. *E. coli* (11.8%) and group D streptococci (4.5%) were encountered relatively infrequently. Enteric organisms other than *E. coli* and group D streptococci were isolated from few participants.

*G. vaginalis* was recovered from vaginal cultures of about one third of the participants. The role of this

organism as a cause of vaginitis is controversial. Some investigators are convinced of its pathogenicity<sup>12, 13</sup> while others are somewhat skeptical.<sup>14, 15</sup> In this population, we were unable to associate colonization with *G. vaginalis* with an abnormal vaginal discharge, either as reported by the patient or as observed by the examining gynecologist. We recovered *G. vaginalis* from 16 (29%) of 56 sexually inexperienced women and found that sexual experience had only a modest influence on colonization. These data have been reported in detail elsewhere.<sup>2</sup> Whatever its role, if any, in vaginitis, it is clear that *G. vaginalis* is an extremely common vaginal organism that can be recovered from normal, non-symptomatic, sexually inexperienced women. Thus, the isolation of *G. vaginalis* from an individual patient is in and of itself of no clinical significance.

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# Mechanism of luteolysis: Effect of estradiol and prostaglandin $F_{2\alpha}$ on corpus luteum luteinizing hormone/human chorionic gonadotropin receptors and cyclic nucleotides in the rhesus monkey

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Recent studies from our laboratory suggest that estrogen-induced luteolysis in the primate may be mediated through intraovarian synthesis of prostaglandin  $F_{2\alpha}$  ( $PGF_{2\alpha}$ ). To elucidate further the mechanism of luteolysis, normally cycling rhesus monkeys received intra-corpus luteum (CL) injections of estradiol (100  $\mu$ g),  $PGF_{2\alpha}$  (500  $\mu$ g), or the appropriate vehicles on the seventh day after the preovulatory estradiol surge. Peripheral vein blood was drawn at 30-minute intervals for 6 hours for progesterone and luteinizing hormone (LH) assays. Five hours after intra-CL injection, the CL was excised and the LH/human chorionic gonadotropin (hCG) receptor-binding activity and cyclic guanosine monophosphate (cGMP) and cyclic adenosine monophosphate (cAMP) were measured.  $PGF_{2\alpha}$  significantly ( $p < 0.05$ ) lowered progesterone within 1 hour, while the time required for estradiol to lower progesterone significantly was 3.5 hours; there was no significant change in LH. Estradiol and  $PGF_{2\alpha}$  significantly ( $p < 0.05$ ) decreased the LH receptor-binding capacity at 5 hours, without any change in the binding affinity. Also,  $PGF_{2\alpha}$  significantly ( $p < 0.05$ ) increased cGMP in the CL, while cAMP remained unchanged; estradiol treatment resulted in a significant ( $p < 0.05$ ) increase in cAMP with no change in cGMP. This study suggests that estradiol and  $PGF_{2\alpha}$  cause a decrease in progesterone secretion by a loss of the LH/hCG receptor and the  $PGF_{2\alpha}$  may act further through the cGMP system. (AM. J. OBSTET. GYNECOL. 139:134, 1981.)

SYSTEMIC AND LOCAL (intraluteal) administration of estrogen during the luteal phase of the menstrual cycle has been shown to cause luteal regression in macaques and women.<sup>1-5</sup> It has also been reported that estrogen administration causes an increase in prostaglandin F ( $PGF$ ) measured in the uterine vein in sheep or in the

peripheral circulation of monkeys.<sup>1</sup> Since prostaglandin  $F_{2\alpha}$  is luteolytic in most mammalian species, a causal relationship between estrogen, prostaglandin, and luteal regression in primates has been postulated. We observed recently in our laboratory a significant increase of  $PGF$  in the ovarian vein of the rhesus monkey

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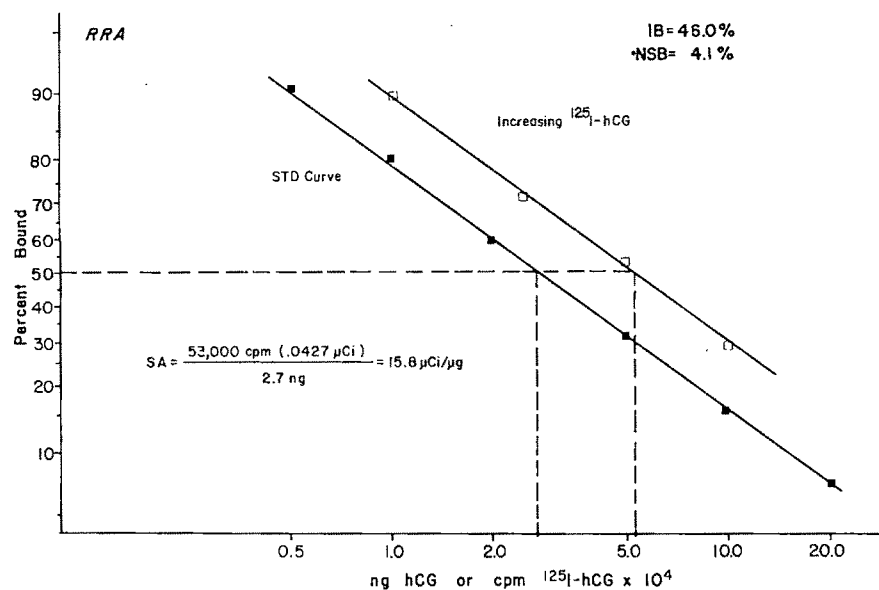


**Fig. 1.** An inhibition curve was generated by adding, in duplicate, sequential dilutions of hCG and membrane preparations (*STD Curve*). In addition, sequential increasing quantities of  $^{125}\text{I}$ -hCG were added to tubes containing membrane preparations ( $^{125}\text{I}$ -hCG). Specific activity was calculated by dividing the counts per minute obtained at a bound/free ratio of 50% by the quantity of hCG that displaced 50% of  $^{125}\text{I}$ -hCG in the inhibition curve. IB = Initial binding. NSB = Nonspecific binding.

after intravenous infusion of estradiol with a concomitant decrease in progesterone without a change in the peripheral luteinizing hormone (LH) levels.<sup>2</sup> This effect could be blocked with indomethacin, a potent inhibitor of prostaglandin synthetase.<sup>3</sup>

The present study was undertaken to elucidate further the mechanism by which estrogen and  $\text{PGF}_{2\alpha}$  cause a decrease in progesterone secretion in the primate. It was postulated that estradiol decreases progesterone production through a prostaglandin-mediated local, intraovarian or intra-corpus luteum pathway. To test the hypothesis, estradiol and  $\text{PGF}_{2\alpha}$  were administered locally into the corpus luteum of the *Macaca mulatta* in an acute surgical experiment. The change in peripheral progesterone and LH levels as well as the number of LH/human chorionic gonadotropin (hCG) receptor sites and the cyclic adenosine monophosphate (cAMP) and cyclic guanosine monophosphate (cGMP) content of the excised corpus luteum were measured.

#### Material and methods

Rhesus monkeys weighing 5 to 7 kg and individually housed were considered suitable for the experiment after at least one normal menstrual cycle had been documented by serial measurements of estradiol and progesterone in the peripheral blood. Operation was performed on the seventh day after the preovulatory estradiol rise.

**Surgical technique.** With the animals under halo-

thane anesthesia, laparotomy was performed with the use of sterile techniques. The corpus luteum (CL)-bearing ovary was identified and the CL was injected slowly, by means of a Hamilton syringe with a 26-gauge needle to prevent leakage. Prior to the anesthesia and intra-CL injection a blood sample was taken from an indwelling catheter located in the saphenous vein to determine the effect of anesthesia and surgical stress on progesterone and LH secretion.

**Radioimmunoassays (RIAs).** After the intra-CL treatment, blood samples were drawn at 30-minute intervals for 6 hours, for later analysis of progesterone and LH by radioimmunoassay techniques previously described.<sup>2</sup>

Five hours after the intra-CL injection, the CL was enucleated (CLEX), divided, and weighed for both receptor determination and cyclic nucleotide assay.

The portion of tissue used for cyclic nucleotide assay was immediately frozen at  $-70^\circ\text{C}$  until analysis.

**Gonadotropin-binding studies.** The numbers of unoccupied LH receptor sites were estimated by Scatchard analysis with the use of  $^{125}\text{I}$ -hCG. A total of  $2\text{ }\mu\text{g}$  of highly purified hCG (Canfield, CR-119, 11,600 IU/mg) was iodinated with 1 mCi of  $^{125}\text{I}$ -Na, according to the Chloramine-T method of Greenwood and Hunter,<sup>6</sup> as modified by Hichens and associates.<sup>7</sup> The identity of the labeled hormone was demonstrated by obtaining parallel displacement curves of increasing amounts of labeled hCG only with increasing quantities of un-

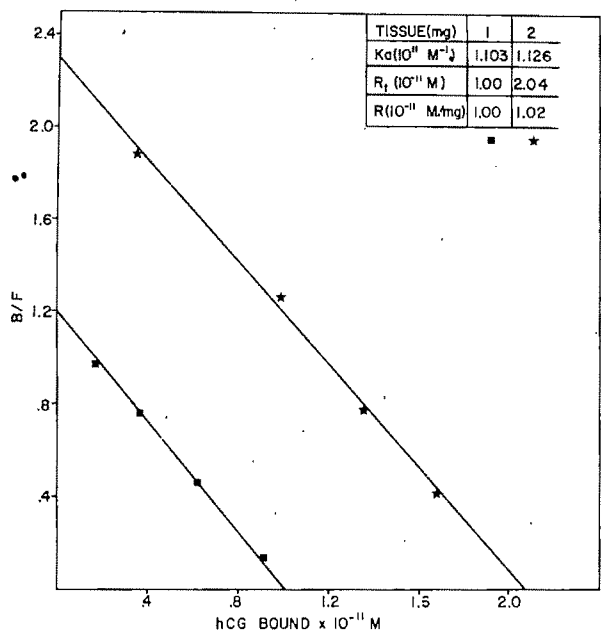


Fig. 2. Effect of concentration of protein on the estimation of the number of receptor sites. Two concentrations of membrane were incubated with <sup>125</sup>I-hCG. Bound radioactivity was separated from free radioactivity and the data were subjected to Scatchard analysis.

Table I. Peripheral LH levels assayed at -1, 0, and 5 hours of treatment. No significant change in LH was observed

| Treatment                   | LH (µg/ml)*   |                  |           |
|-----------------------------|---------------|------------------|-----------|
|                             | Preanesthesia | Pre-CL injection | DEX       |
| Tromethamine buffer (N = 4) | 1.6 ± 0.2†    | 1.8 ± 0.3        | 1.9 ± 0.3 |
| PGF <sub>2α</sub> (N = 3)   | 2.1 ± 0.5     | 1.8 ± 0.3        | 1.9 ± 0.2 |
| Sesame oil (N = 2)          | 1.9 ± 0.4     | 1.7 ± 0.2        | 2.0 ± 0.3 |
| Estradiol (N = 4)           | 1.8 ± 0.3     | 1.9 ± 0.2        | 1.7 ± 0.3 |

\*Expressed as LER 1909-2 equivalents.  
†Mean ± SEM.

labeled hCG in the radioreceptor assay with the CL membrane (Fig. 1). The same assay, as well as the RIA with specific antiserum to the β-subunit of hCG (BIO-RIA, Montreal, Canada), was used to estimate the specific activity, as described by Diekman and associates.<sup>8</sup> Both methods yielded almost identical estimates: 15.4 and 14.6 and 9.8 and 10.3 mCi/mg for the two batches prepared (radioreceptor assay and RIA, respectively).

The excised CL was placed in ice-cold 0.04M tromethamine-hydrochloric acid, 0.005M magnesium sulfate buffer, pH 7.4, and homogenized in a Teflon glass grinder. The homogenate was drawn through a 26-gauge needle and filtered through four layers of

gauze before centrifugation at 2,000 × g for 20 minutes. The supernatant was then discarded; the pellet was resuspended in ice-cold tromethamine buffer and the centrifugation was repeated. After three centrifugations for 20 minutes each, the final pellet was resuspended in the desired amount of tromethamine buffer and frozen at -70° C until analyzed.

Thawed aliquots of 0.2 ml (containing 1 to 4 mg of tissue) were incubated overnight at room temperature with the labeled <sup>125</sup>I-hCG and increasing amounts of unlabeled hCG in a final volume of 0.5 ml. Nonspecific binding was determined by using 100 IU of hCG (Pregnyl). Following the incubation, a suspension of Celite (1.25 mg) in 0.2% bovine serum albumin-tromethamine buffer was added to each tube before centrifugation at 2,000 × g. The radioactivity of the receptor Celite pellet was measured in a gamma counter. The binding data of <sup>125</sup>I-hCG to the 2,000 × g subcellular fraction were plotted after the nonspecific binding was subtracted; the data were quantitated by Scatchard analysis. Maximum binding with receptors in the excess was 50% to 60%. No correction was made for incubation damage to the hormone or receptors. To test whether the concentration of receptors would alter the estimation of the number of receptor sites by Scatchard analysis, membranes equivalent to 1 and 2 mg of tissue were incubated as described. After analysis, the concentration of receptors per milligram of tissue was identical at the two concentrations of tissue tested and the affinity constant did not change (Fig. 2). When frozen membrane preparations were analyzed up to 5 months apart, no change was seen in the binding affinity or capacity of the preparations.

**Cyclic nucleotide RIA.** cAMP and cGMP were determined by RIA as described by Steiner.<sup>9</sup> With the use of RIA kits purchased from Becton, Dickinson & Co., the thawed tissue was extracted with trichloroacetic acid and free and bound nucleotides were separated with the use of 60% ammonium sulfate. All tissue preparations were quantitated in one RIA (in triplicate): The intra-assay coefficients of variation were 13% for the cGMP and 3% for the cAMP. Recoveries through the method were 65% to 68% for cGMP and 75% to 89% for cAMP.

**Experimental groups.** There were four experimental groups. The first group of four monkeys received 100 µg of estradiol in 100 µl of sesame oil into the CL. The second group of two animals received 100 µl of sesame oil vehicle and served as controls for the estradiol group. In the third group of three animals, 500 µg of PGF<sub>2α</sub> in 100 µl of tromethamine buffer was injected into the CL. This dose is equivalent to 373.1 µg of PGF<sub>2α</sub>. Four animals received 100 µl of tromethamine buffer and served as controls for the PGF<sub>2α</sub> group.



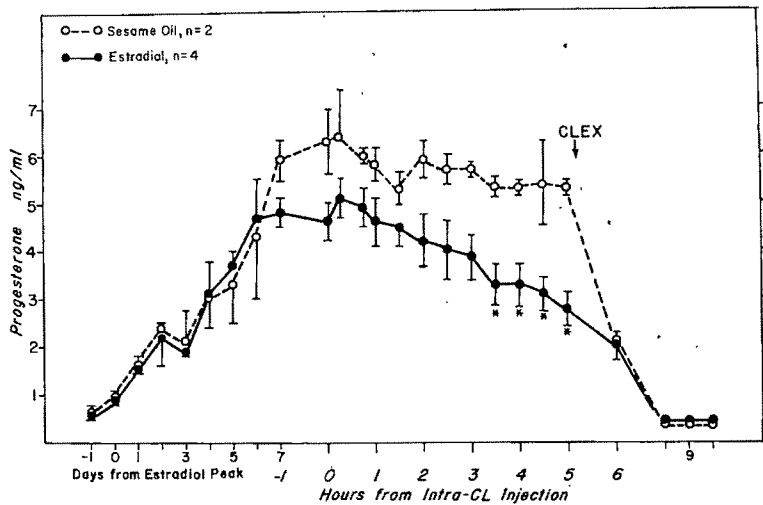


Fig. 3. Mean  $\pm$  SE of plasma progesterone in monkeys before and after intra-CL injection of either sesame oil (100  $\mu$ l) or estradiol (100  $\mu$ g/100  $\mu$ l). Note that there was a significant (\* $p$  < 0.05) decrease in progesterone 3.5 hours after intra-CL injections that was sustained until CLEX.

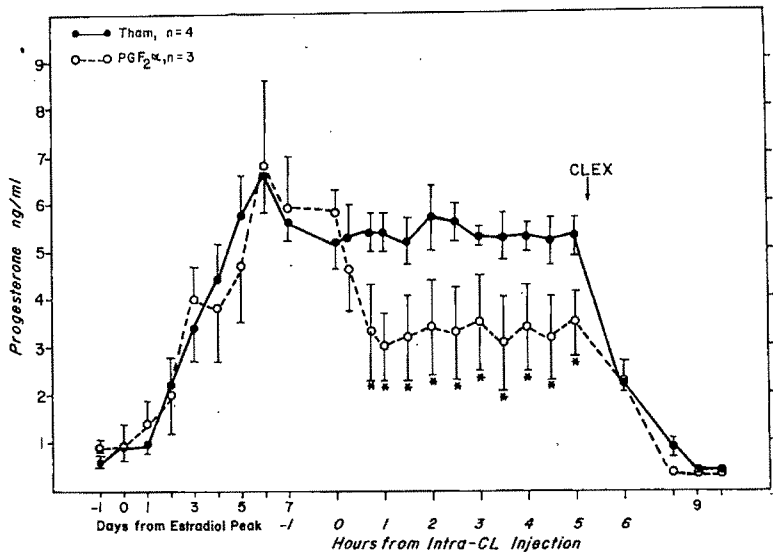


Fig. 4. Mean  $\pm$  SE of plasma progesterone in monkeys before and after intra-CL injection of either tromethamine buffer (*Tham*) (100  $\mu$ l) or PGF<sub>2 $\alpha$</sub>  (500  $\mu$ g/100  $\mu$ l). Note that there was a significant (\* $p$  < 0.05) decrease in progesterone 40 minutes after intra-CL injection that was sustained until CLEX.

**Statistical analysis.** The statistical significance of the plasma progesterone and LH data was assessed by means of the one-way analysis of variance with repeated measurements. The CL gonadotropin-binding studies and cyclic nucleotide results were analyzed by Student's *t* test. A *p* value of less than 0.05 was considered to be significant.

### Results

**Effect of estradiol and PGF<sub>2 $\alpha$</sub>  on the peripheral progesterone and LH level.** The anesthesia and surgi-

cal stress did not affect progesterone or LH secretion, as shown in the animals receiving the vehicles only (Figs. 3 and 4). A significant decrease in progesterone occurred at 3.5 hours after the estradiol injection and continued to decline gradually until the CLEX (Fig. 3). There was no significant change in LH (Table I). The significant depression of progesterone after PGF<sub>2 $\alpha$</sub>  administrations (Fig. 4) was observed at 1 hour following the CL injection. The progesterone level remained significantly lower through the time of CLEX. Once again, LH was not significantly affected (Table I). Fig. 5

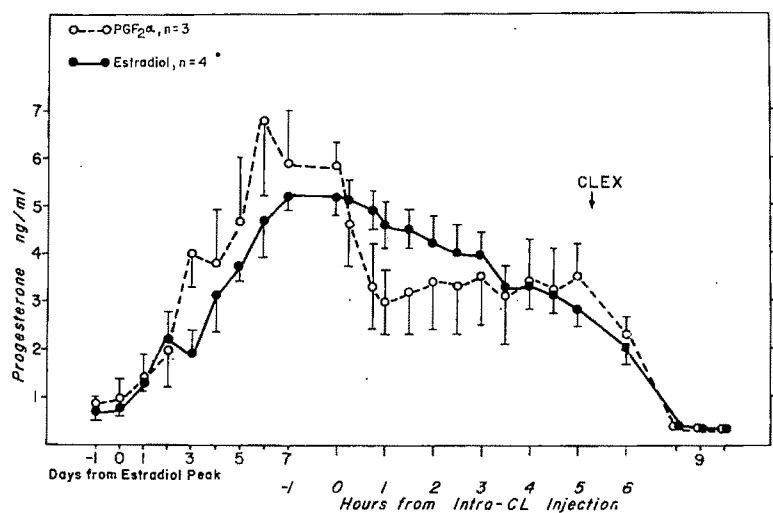


Fig. 5. Mean  $\pm$  SE of plasma progesterone in monkeys treated with estradiol (100  $\mu$ g) or PGF<sub>2 $\alpha$</sub>  (500  $\mu$ g). Note that PGF<sub>2 $\alpha$</sub>  caused an immediate decline of progesterone while estradiol treatment resulted in a steady decline.

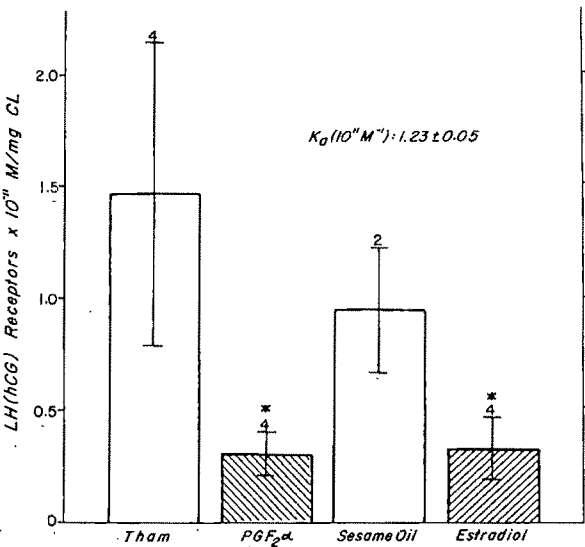


Fig. 6. Mean  $\pm$  SE of LH (hCG) receptor-binding capacity after treatment with PGF<sub>2 $\alpha$</sub> , estradiol, and their appropriate vehicles. Both PGF<sub>2 $\alpha$</sub>  and estradiol treatment resulted in a significant (\* $p < 0.05$ ) decrease in LH (hCG) receptors without any change in the binding affinity.

superimposes the results of estradiol and PGF<sub>2 $\alpha$</sub>  treatments. It can be seen that the action of PGF<sub>2 $\alpha$</sub>  occurs very rapidly when compared to that of estradiol; however, estradiol treatment results in a continued decline in progesterone.

**Effect of estradiol and PGF<sub>2 $\alpha$</sub>  on LH-binding kinetics.** Fig. 6 shows the results of estradiol and PGF<sub>2 $\alpha$</sub>  administration on LH/hCG receptor-binding capacity. Both estradiol and PGF<sub>2 $\alpha$</sub>  significantly ( $p < 0.05$ ) decreased the binding capacity 5 hours after the intra-CL

injection without any change in the binding affinity. **Effect of estradiol and PGF<sub>2 $\alpha$</sub>  on CL cyclic nucleotides.** PGF<sub>2 $\alpha$</sub>  significantly increased cGMP while cAMP remained unaltered. Estradiol caused a significant increase in cAMP with no change in cGMP (Fig. 7).

**Comment**

The results of this study clearly indicate that intra-luteal administration of estradiol or PGF<sub>2 $\alpha$</sub>  causes a significant depression in CL function, without any change in peripheral blood levels of LH. The rapid decrease in progesterone following PGF<sub>2 $\alpha$</sub> , compared to the delayed, steady decrease after estradiol injection, and our previous findings that estradiol action could be blocked by the prostaglandin synthesis inhibitor indomethacin<sup>2, 3</sup> suggest that estradiol action may be prostaglandin mediated. Estradiol probably caused continued synthesis of ovarian PGF<sub>2 $\alpha$</sub> ,<sup>2</sup> while injected PGF<sub>2 $\alpha$</sub>  directly affected luteal steroidogenesis. It could be argued that the doses of estradiol and PGF<sub>2 $\alpha$</sub>  used in this experiment were pharmacologic rather than physiologic. Butler and associates<sup>10</sup> reported the concentration of estradiol in the CL of rhesus monkeys during the late luteal phase to be approximately 15 ng/gm of tissue. No data are available as to the concentration of PGF<sub>2 $\alpha$</sub>  in the CL of this species. However, Shutt and associates<sup>11</sup> have reported the concentrations of PGF in the human CL to range between 10 and 46 ng/gm of tissue. Since the concentrations of estradiol and PGF were not measured in our experiment, the possibility exists that the doses used may have been pharmacologic. However, after the injection of 100  $\mu$ g of estradiol in



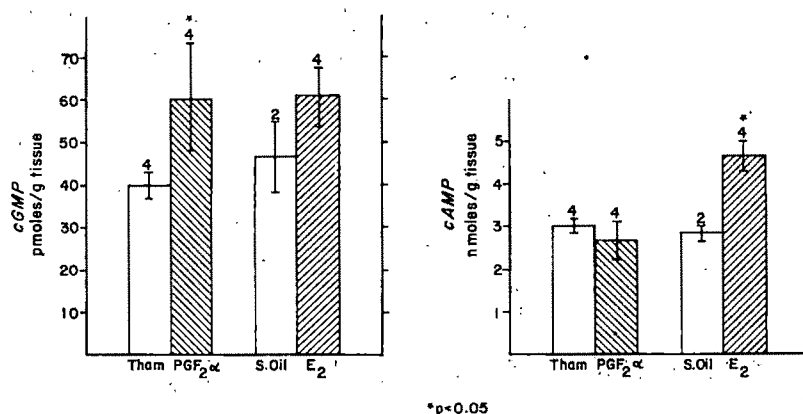


Fig. 7. Mean  $\pm$  SE of CL cGMP (left) and cAMP (right) levels after treatments with PGF<sub>2α</sub>, estradiol, and their vehicles. PGF<sub>2α</sub> significantly increased cGMP and estradiol significantly increased cAMP.

the ovarian stroma of rhesus monkeys, Karsch and Sutton<sup>12</sup> noted a doubling in the plasma estradiol level to a mean of 130 pg/ml. We observed a similar doubling in plasma estradiol after the intravenous infusion of 1 ng of estradiol per minute for 3 hours in rhesus monkeys during the luteal phase.<sup>2</sup> As noted, such infusion was associated with a decrease in progesterone level without change in peripheral LH levels. These data lend support to the physiologic relevance of the results obtained in the present study.

Since LH plays a fundamental role in the formation and maintenance of the CL, its role may be permissive in that major alterations in luteal function are not directly attributable to altered secretion of this gonadotropin. Our understanding of regulation of steroidogenesis in the CL is seen in light of current concepts of peptide hormone research. According to such concepts, LH binds to receptors in the luteal cell membrane. Such binding results in the activation of the adenyl cyclase system that generates the second messenger, cAMP. The newly formed cAMP is transported by a carrier protein to an inactive protein kinase. With the binding of the cAMP to the protein kinase, its catalytic subunit becomes active, thus triggering the observed hormonal effect. In the case of the luteal cell, progesterone production would be increased.

Spontaneous luteolysis is not related to decreased LH secretion and probably results from an inability of the CL to respond to LH. Support for this hypothesis is available from experiments in which luteal LH receptor kinetics were determined in pseudopregnant rats<sup>13</sup> and in women<sup>14</sup>; it was reported that as progesterone levels declined during the end of the cycle, the ability of the luteal tissue to bind labeled LH also decreased. In the present study, the corpora lutea were excised 5 hours after intraluteal injection of PGF<sub>2α</sub> or estradiol, and there was a significant reduction in the number of

LH receptors. However, the direct causal relationship between the LH receptor reduction and the decrease in progesterone observed in our experiment remains speculative, since the LH receptors were measured a few hours after blood progesterone levels had fallen. In the rat<sup>15</sup> and the ewe,<sup>16</sup> the PGF<sub>2α</sub>-induced decrease in progesterone secretion precedes any change in the LH receptors measured *in vitro*. However, *in vivo* experiments in pseudopregnant rats showed that PGF<sub>2α</sub> rapidly reduced the uptake of <sup>125</sup>I-hCG by corpora lutea at the time of the progesterone decrease.<sup>17</sup>

Contrary to the above findings, Thomas and associates<sup>18</sup> observed in the rat that PGF<sub>2α</sub>-induced luteolysis was not associated with a decreased uptake of labeled hCG or loss of gonadotropin receptors when measured at 1 hour but that the rapid action of PGF<sub>2α</sub> was due to inhibition of LH-dependent production of cAMP. The time and species difference between this study and that of Thomas and associates<sup>18</sup> may explain these opposing results. In our study no change in cAMP 5 hours after PGF<sub>2α</sub> was noted.

Current concepts of the luteolytic actions of PGF<sub>2α</sub> favor both a rapid and a slow component. The rapid component involves a block of gonadotropin uptake *in vivo* by an unexplained mechanism<sup>17</sup> and a direct block of adenyl cyclase activation by the LH receptor complex.<sup>18</sup>

The slow component involves the eventual loss of LH receptor-binding activity.<sup>16</sup> The results of the present study are in agreement with this LH receptor loss, which in the primate may occur as early as 5 hours after administration of PGF<sub>2α</sub>.

However, the lack of decrease of cAMP is difficult to reconcile with the above-cited concepts. It is possible that the increase in luteal cGMP after PGF<sub>2α</sub> treatment noted in our experiment may have modulated the action of cAMP in the luteal cell,<sup>19</sup> resulting in the overall

decline in progesterone production. There is ample evidence in the literature that the relative level of cAMP to cGMP may be important in determining the direction of cellular metabolism.<sup>20</sup>

The increase in luteal cAMP after estradiol administration suggests that the site of estrogen action might occur at some step beyond adenyl cyclase activation. Williams and associates,<sup>21</sup> using human luteal cells in suspension, noted that estradiol inhibited hCG-induced progesterone secretion but not cAMP accumulation; these authors also observed that estradiol blocked the action of dibutyl cAMP on progesterone synthesis, suggesting a direct effect of estradiol on the  $3\beta$ -hydroxysteroid dehydrogenase and  $\Delta 4,5$ -isomerase enzymes. We do not preclude the possibility that estradiol could act directly on this enzyme system or other post-cAMP-mediated events while also inducing endogenous synthesis of PGF<sub>2 $\alpha$</sub>  to reduce LH receptor activity.

Although decreased progesterone production in an acute 5-hour experiment cannot be equated to luteolysis, the acute preparation utilized here provides a model which may explain the estrogen-induced functional regression of the primate CL. Estradiol and PGF<sub>2 $\alpha$</sub>  directly (not via the pituitary) inhibit progesterone synthesis by the rhesus monkey CL in vivo. The inhibition is accompanied and perhaps caused by a loss of LH receptors and a change in cyclic nucleotides.

We acknowledge Drs. Gayle Orczyk and Gordon Niswender for their gifts of progesterone and LH antisera, and we thank Dr. Leo Reichert, Jr., for the crude rhesus monkey LH standard (LER 1909-2) and the ovine LH (LER 1056-C2) for iodination. We also acknowledge the National Pituitary Agency for supplying the hCG (CR-119) for the receptor-binding studies. The Department of Laboratory Animal Medicine provided excellent animal care and surgical assistance.

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# Deaths associated with laparoscopic sterilization by unipolar electrocoagulating devices, 1978 and 1979

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In 1978 and 1979, two women in the United States were reported to have died from electrical complications following sterilization with unipolar coagulating devices. Both deaths followed apparent bowel injuries occurring at the time of sterilization. Numerous reports have documented the electrical accidents associated with unipolar electrocoagulation. Because unipolar electrocoagulation has greater risk for these complications than alternative sterilization techniques, without proved greater benefits, we question the need for continuing its use in female sterilization. (AM. J. OBSTET. GYNECOL. 139:141, 1981.)

LAPAROSCOPY is used in over one third of female sterilizations in the United States,<sup>1</sup> and in most of these procedures electric current is used to coagulate the fallopian tubes.<sup>2</sup> The majority of coagulations are performed with unipolar devices,<sup>2</sup> which carry the risks of accidental burns to the patient<sup>3</sup> and operator.<sup>4</sup> Through the Center for Disease Control's nationwide surveillance of sterilization-related deaths, we have identified two deaths in the United States in the years 1978 and 1979 that apparently resulted from inadvertent burns to the bowel sustained during sterilization with unipolar devices.

## Case presentations

**Case 1.** A healthy, 41-year-old woman, gravida 6, para 5, abortus 1, underwent laparoscopic tubal steril-

ization via electrocoagulation with a unipolar device. Coagulation, resection, and division of a segment of each tube were performed without apparent incident. The patient was discharged the day after operation.

Twenty-three days later, she returned to the hospital with abdominal pain and evidence of peritonitis. She gave a history of having been constipated for several days prior to admission but of otherwise doing well until shortly before entering the hospital, when she had begun to vomit and experience severe abdominal pain. When seen in the emergency room, the patient was found to be in septic shock.

The following morning, she underwent laparotomy. Purulent material was noted throughout the peritoneal cavity. Although the upper gastrointestinal tract was normal, the small and large bowels were covered by a fibrinous exudate. Multiple adhesions were present and a perforation was noted on the anterior wall of the midportion of the sigmoid colon. A colectomy and diverting colostomy were performed. Histologic review of the 5 cm colectomy specimen revealed that the perforation had occurred at the site of a thermal injury. The patient's condition deteriorated and she died 41 days after laparotomy. Autopsy revealed a right-sided subdiaphragmatic abscess, peritonitis, and a small bowel perforation communicating with a subcutaneous abscess and fistula tract to the skin.

**Case 2.** A healthy, 22-year-old woman, gravida 4, para 4, underwent dilatation and curettage followed by laparoscopic coagulation tubal sterilization with a unipolar device. The curettage proceeded without incident and yielded a scant amount of normal-appearing

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tissue. Electrocoagulation with resection and division of each fallopian tube was performed without apparent difficulty. The woman was discharged on the following day.

Seven days after the operation, the patient was hospitalized because of abdominal pain. She was febrile and had evidence of intestinal obstruction. Antibiotics were initiated, and an emergency laparotomy was performed. Findings included diffuse peritonitis. Although the surgeon believed preoperatively that bowel perforation had occurred, an attempt to identify the perforation site was unsuccessful. The effort was hampered because the bowel was covered with purulent material. No gastroduodenal perforation could be identified and the pelvis appeared entirely normal. Material from the abdominal cavity was cultured, the cavity was irrigated, and then the wound was closed.

After operation, the patient's condition deteriorated and she died 2 days later of septic shock. An autopsy was performed the following day. There were no new findings from those identified at surgery, i.e., no source of the peritonitis could be determined. Cultures of the abdominal cavity material grew *Enterobacter cloacae*, *Citrobacter freundii*, *Escherichia coli*, *Klebsiella pneumoniae*, *Clostridium perfringens*, and *Bacteroides* spp.

### Comment

The two deaths apparently resulted from the small bowel injury following electrical accidents with unipolar coagulating devices. The colectomy specimen from Case 1 confirmed that a bowel burn had occurred. In Case 2, bowel perforation is strongly implicated by the following: the presence of a profuse, purulent discharge covering the bowel; the absence of gastroduodenal perforation or pelvic inflammatory disease; and the growth of fecal flora in the cultures of material from the peritoneal cavity. While some of the organisms cultured might be recovered following pelvic inflammatory disease alone,<sup>5</sup> it is very unlikely that all these organisms would have been recovered in the absence of a bowel perforation.

Most electrical injuries to the bowel are unrecognized at the time of occurrence.<sup>3</sup> In both cases reported here, the tubal ligation was thought to have proceeded without complications. Patients with unrecognized bowel injuries generally present 3 to 7 days after injury with complaints of fever and abdominal pain.<sup>3</sup> Reported intervals from time of occurrence of injury to onset of symptoms vary from 18 hours to 14 days.<sup>3</sup> Onset of symptoms in Case 2 was consistent with this interval, but the patient in Case 1 did not present until much later. Both patients presented with fever and abdominal pain. As in Cases 1 and 2, persons who present after bowel rupture are often critically ill on admission.<sup>6</sup>

### Prevention of deaths caused by thermal bowel in-

**jury.** With unipolar instruments, coagulation is carried out with electric current that proceeds through the operating instrument to the forceps (active electrode). The current then coagulates the tube and courses through the patient's body to a grounding plate (return electrode) on the patient's skin. Therefore, the patient is an integral part of the electric circuit.

Such an electric system has resulted in major accidents. The most frequent serious complication reported has been thermal injury to the bowel.<sup>3</sup> Over 100 such cases following unipolar coagulation have been reported.<sup>3, 7-9</sup> In its most recent membership survey, the American Association of Gynecologic Laparoscopists reported the incidence of bowel burns with unipolar instruments to be 0.5 per 1,000 cases,<sup>8</sup> a rate similar to that found in the national survey conducted in the United Kingdom.<sup>9</sup>

Since most bowel injuries with unipolar devices are unrecognized at the time of operation, the reasons for their occurrence can only be speculated. Many of the reported burns may have resulted from inadvertent touching of the bowel by activated forceps.<sup>3</sup> Another theoretical cause is arcing of sparks within the abdomen. Because current used to coagulate the fallopian tube must course through the patient's body to reach the return electrode, it is possible that use of high-voltage current may result in sparks being generated, causing injury to the adjacent bowel.<sup>10, 11</sup> Other bowel burns may be explained by the property of capacitance. If nonconductive laparoscopes are used in unipolar systems, it is possible for the laparoscope to store current, thus becoming a capacitor able to cause burns if it touches the bowel unexpectedly.<sup>11</sup>

The unipolar system also allows the occurrence of electric accidents other than bowel injuries. Where contact between the grounding plate and the patient is inadequate, the current delivered by the active electrode may exit to a part of the patient which is better grounded, causing skin burns.<sup>12</sup> Burns of the abdominal wall result when current is carried to the ground plate via the trocar sheath that surrounds the cautery instrument. By 1975, at least 25 such abdominal wall burns had been reported.<sup>3</sup> Burns to the face and hands of the operator occurring by similar mechanisms also have been reported.<sup>4</sup>

Some investigators have urged the concept of bipolar coagulation to reduce the risk of burns.<sup>12, 13</sup> In 1974, Rioux (see Rioux and Cloutier<sup>10</sup>) pioneered an instrument to perform sterilization by this technique. With bipolar cautery, the jaws of the grasping forceps serve as both the active and return electrodes, so that current passes between the two prongs, burning only the tissues grasped. This technique markedly reduces the risk of



bowel injuries. Only one case of thermal bowel injury has been reported with the use of the bipolar system.<sup>14</sup>

The introduction of mechanical devices for tubal occlusion offered nonelectric alternatives to unipolar systems for sterilization by laparoscopy. The spring clip developed by Hulka and Clemens (see Hulka and associates<sup>15</sup>) and the silicone rubber band pioneered by Yoon (see Yoon and King<sup>16</sup>) were introduced at approximately the same time as the bipolar technique. These devices require no use of electric current within the patient's body.

Which technique of laparoscopic sterilization is the safest and most effective remains unclear. Currently there are limited data on the comparative risks and benefits of the various devices. When an electric technique is chosen, the operator must decide whether to use unipolar or bipolar systems. Available reports<sup>3, 4, 6-14</sup> and theoretical considerations suggest that unipolar coagulation has greater risks for injury than bipolar coagulation. Other reports indicate that the unipolar technique is not more effective in producing sterility. In fact, the AAGL's most recent membership survey indicates that while intrauterine pregnancy rates are approximately the same with unipolar and bipolar cau-

tery, there is a possible increased risk of ectopic pregnancy when sterilization is performed with unipolar devices.<sup>2</sup>

The Center for Disease Control's nationwide sterilization mortality surveillance effort is designed to determine why women die from sterilization procedures and to identify ways to prevent such deaths. A review of the laparoscopy-associated deaths identified to date indicates that the only deaths related to the method of tubal occlusion per se occurred with a unipolar device. A similar finding has been recently reported from the United Kingdom where three deaths were identified in nearly 30,000 laparoscopic sterilization procedures. Of these three deaths, only one was associated with the instrument used for sterilization; the death followed a bowel burn sustained with a unipolar device.<sup>9</sup> Because unipolar electrocoagulation apparently has a greater risk of electric accidents than bipolar coagulation, without any demonstrable greater efficacy in producing sterility, we question the need for continuing its use in female sterilization.

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# Cryosurgical treatment of cervical intraepithelial neoplasia with follow-up of five to eight years

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During 1972 to 1974, 181 women of different ages with cervical intraepithelial neoplasia were treated with cryosurgery. Of these, 170 have now been observed for 5 to 8 years. Treatment failures total 16%, but when the different age groups are considered the results are seen to be much better in young women less than 30 years of age, with only a 12% rate of failure, whereas older women have almost twice as many failures. One patient, 45 years of age, developed an early invasive cancer, but in her case pretreatment diagnosis may have been incorrect. Our conclusions are that cryosurgery is an easy and effective method of treating cervical intraepithelial neoplasia in young women, provided that the diagnosis prior to therapy is correct. Furthermore, we stress that after treatment the status of the patient must be checked with vaginal smears over a long period and also that at least one endocervical curettage and directed biopsies of the vaginal cervix should be done as a control. (AM. J. OBSTET. GYNECOL. 139:144, 1981.)

IT IS NOW GENERALLY AGREED that dysplasia and carcinoma in situ of the cervix in many cases develop into invasive cancer.<sup>1</sup> This knowledge emphasizes the importance of diagnosing different stages of cervical intraepithelial neoplasia as early as possible and then adopting an appropriate treatment.

Historically the therapy has been exclusively operative, either conization or hysterectomy. The introduction of vaginal cytology, however, has increased the number of diagnosed cervical intraepithelial neoplasias, especially in young women,<sup>2</sup> and these patients, many of whom have not completed their families, need an alternative to surgery with its known complications on fertility and childbearing.<sup>3</sup> During the last 15 years different conservative methods have been used, e.g., electrocoagulation,<sup>4</sup> cryosurgery,<sup>5-7</sup> and more recently laser,<sup>8</sup> with varying results. The most well-established of these is cryosurgery, which has been used at our clinic since 1972. This study is a review of results from a 5- to 8-year follow-up of 170 women treated

Table I. Age of patients treated with cryosurgery

| Age (years) | No. of patients |
|-------------|-----------------|
| ≤19         | 6               |
| 20-29       | 95              |
| 30-39       | 47              |
| 40-49       | 18              |
| 50-59       | 3               |
| 60-69       | 1               |
| Total       | 170             |

cryosurgically for dysplasia or carcinoma in situ of the cervix.

### Material and methods

This study includes patients with cervical dysplasia or carcinoma in situ treated by cryosurgery at the Department of Gynecologic Oncology, University Hospital, Uppsala, during 1972 to 1974. All of them were initially referred to the clinic because of repeated abnormal vaginal smears. For each patient the pretreatment histopathologic diagnoses were established by cervical biopsies and endocervical curettage. The vaginal cervix and the cervical channel were treated separately with instruments especially designed for these two anatomic structures, and liquid nitrogen was used as the refrigerant, giving a temperature of -170° to -190° C. For each locale the freezing was performed in

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Table II. Results of treatment with cryosurgery

| Pretreatment diagnosis | No. | Follow-up diagnosis |           |                   |                    |
|------------------------|-----|---------------------|-----------|-------------------|--------------------|
|                        |     | Negative            | Dysplasia | Carcinoma in situ | Invasive carcinoma |
| Dysplasia              | 40  | 36 (90%)            | 2         | 2                 |                    |
| Carcinoma in situ      | 130 | 106 (82%)           | 8         | 15                | 1                  |
| Total                  | 170 | 142 (84%)           | 10        | 17                | 1                  |

two sessions, each 30 seconds long and with a defrosting period of 3 minutes between sessions. Initially, some patients were treated in a slightly modified way but after November, 1972, all patients were treated alike. A more detailed description of the freezing technique will be presented elsewhere.

The cryosurgical treatment was performed on an outpatient basis with the use of local anesthesia (paracervical block). The patients were allowed to return to work immediately or on the day following the treatment. After cryosurgery, vaginal smears were taken on all patients at 1 to 3, 6, and 12 months. Thereafter the patient was examined every year. If a patient showed persistent cytologic atypia, cervical biopsies and endocervical curettage were performed. If these revealed dysplasia or carcinoma in situ, the treatment was considered a failure. Most of these patients were then subjected to more definitive therapy, e.g., conization or hysterectomy. The following classification was used: Carcinoma in situ included carcinoma in situ and severe dysplasia comparable with carcinoma in situ; dysplasia included mild, moderate, and severe dysplasia (not comparable with carcinoma in situ).

Results

From June, 1972, to December, 1974, a total of 181 patients with dysplasia or carcinoma in situ of the cervix were treated with cryosurgery. For different reasons nine of these were not accessible for follow-up, and another two refused to come to our clinic for control smears. The remaining 170 patients have now been under observation for 5 years or more. Their ages ranged from 18 to 60 years with the majority between 20 and 29 years of age at the time of cryosurgery (Table I). As seen in Table II, the pretreatment diagnosis was dysplasia of different degrees in 40 cases and carcinoma in situ in 130. The overall recovery rate was 84% with a slightly higher figure in the dysplasia group compared to that of carcinoma in situ. At the follow-up examinations 28 patients were found to have residual atypia of the cervix. Ten had dysplasia; 17 had carcinoma in situ, and one patient had an early invasive cancer. The latter patient was a 45-year-old woman who was treated because of carcinoma in situ. How-

Table III. Time between cryosurgery and diagnosis and treatment of patients with failure

| Time since cryosurgery (months) | First abnormal vaginal smear (No. of patients) | Biopsies and endocervical curettage and/or conization or hysterectomy (No. of patients) |
|---------------------------------|------------------------------------------------|-----------------------------------------------------------------------------------------|
| ≤12                             | 24                                             | 15                                                                                      |
| 13-24                           | 3                                              | 9                                                                                       |
| 25-36                           | 1                                              | 1                                                                                       |
| 37-48                           | 0                                              | 0                                                                                       |
| 49-60                           | 0                                              | 3                                                                                       |

Table IV. Failures in relation to age at time of cryosurgery

| Age at time of cryosurgery (years) | No. of patients treated | No. of patients with failure | Percentage of failures |
|------------------------------------|-------------------------|------------------------------|------------------------|
| ≤29                                | 101                     | 12                           | 12                     |
| ≥30                                | 69                      | 16                           | 23                     |

ever, invasive cancer was diagnosed only 2 months after cryosurgery, which may indicate an incorrect pretreatment diagnosis.

In almost every case of treatment failure the first abnormal vaginal smear appeared within 1 year (Table III). In fact, as many as 18 of the 28 patients had the first positive smear 1 to 3 months after cryosurgery. As shown in the same table, there was often a delay, for reasons unknown, in further treatment of the patients with failure and some women were not correctly treated until some years after the appearance of abnormal smears. However, none of them developed invasive cancer.

When the treatment failure rate is examined in relation to age at the time of cryosurgery (Table IV), it is seen that there were twice as many failures in the older group as in the younger (23% and 12%, respectively). Eight of the women who had recurrent atypia of the cervix were treated by directed punch biopsies and endocervical curettage; five had a second cryosurgery treatment, and 10 underwent conization (Table V). In another four patients a hysterectomy was performed and, in accordance with our routines, the woman with

Table V. Treatment of patients with failure

| Diagnosis after treatment failure | Treatment (No. of patients)               |                               |                     |              |                                       |
|-----------------------------------|-------------------------------------------|-------------------------------|---------------------|--------------|---------------------------------------|
|                                   | Punch biopsies and endocervical curettage | Second cryosurgical treatment | Surgical conization | Hysterectomy | Hysterectomy and radiologic treatment |
| Dysplasia                         | 8                                         | 1                             | 1                   |              |                                       |
| Carcinoma in situ                 |                                           | 4                             | 9                   | 4            |                                       |
| Invasive carcinoma                |                                           |                               |                     |              | 1                                     |

the early invasive cancer was treated radiologically before hysterectomy. Of the 28 with failure, 12 were initially subjected to a second cryosurgery treatment. The results were very disappointing as only five were cured (Table V). Additional therapy (directed biopsies, conization, or hysterectomy) was required in the remaining seven before the vaginal smears were negative. After cryosurgery almost every woman complained of a profuse discharge for 2 to 4 weeks but apart from that there were very few other complications. One woman had severe salpingo-oophoritis and was hospitalized for 10 days. Two other patients were observed for a couple of days at the clinic because of moderate bleeding but needed no additional treatment. Finally, nine women were treated with antibiotics because of a suspicion of salpingitis but the diagnoses were never confirmed. No late complication has been reported so far.

Comment

Cryosurgery is today an accepted and well-established therapy for dysplasia and carcinoma in situ of the cervix. At our clinic we have now observed 170 women treated with cryosurgery 5 to 8 years ago. The failure rate in the whole group is 16% and this result is comparable with results reported from other clinics<sup>5, 7</sup> and also with results reported with cold-knife conization.<sup>9</sup> We found that the failure rate for women younger than 30 years of age was only half of that for older women (12% and 23%, respectively). The explanation of this might be the fact that older women more often have lesions high up in the cervical channel where it is difficult to obtain adequate freezing of the epithelium.

Since cryosurgery does not give any material for diagnosis, it is necessary to perform pretherapy directed punch biopsies of the vaginal cervix and endocervical curettage on every patient to rule out the possibility of invasive cancer. Preferably, of course, this should be done with the aid of colposcopy. Among the failures in our material, one patient developed an early invasive cancer. As discussed earlier, we suspect that the pretreatment diagnosis was incorrect and that she

already had invasive carcinoma before cryosurgery. All failures were diagnosed within 3 years after treatment and most of them were diagnosed within the first year. This result is concordant to that found by other investigators.<sup>7</sup> We believe that it is important to realize that a failure represents an insufficiently treated disease and that it should not be regarded as a recurrence. Women who undergo surgical conization because of carcinoma in situ constitute a high-risk group,<sup>10</sup> even after successful therapy, and the same is certainly true for patients treated with cryosurgery. Therefore, these patients should be subjected to a long sequence of annual follow-ups, probably for 10 years or more.

In our clinic cryosurgery is used even if the lesion is localized within the cervical channel. To justify this we have different probes designed especially for the endocervix and ectocervix. In agreement with others,<sup>7</sup> we consider that in all patients treated with cryosurgery an endocervical curettage, and even biopsies of the vaginal cervix, should be performed as a control 1 year after treatment. Of course, this is most important in patients with endocervical lesions. However, this routine was not used from the start of this study and thus the material comprises only about 80 patients, all of them with normal results.

Compared to other methods, cryosurgery has very few complications and the only risk involved is that of salpingitis. Even if very few infections have been reported it is of utmost importance to minimize this risk, as most of the patients treated are young women in whom fertility must be preserved. Consequently, during the past years every patient has been offered preventive antibiotic treatment for 1 week after cryosurgery.

Finally, it is our opinion that cryosurgery is a good therapy when cervical intraepithelial neoplasia is diagnosed in women under 30 years of age. It can be done on an outpatient basis without general anesthesia. The frequency of postoperative complications is very low and the patient can return to work soon after treatment. There is no interference with childbearing or delivery and the risk of reduction of fertility is minimal.



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# Fine-needle aspiration cytology in the management of gynecologic cancer

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The technique and application of fine-needle aspiration cytology in the management of gynecologic cancer are discussed. The authors report 90 cases that have been evaluated by means of fine-needle technique. The technique has been applied both for primary diagnosis and for follow-up evaluation. Among the 90 cases reported, there were two false negatives and one false positive and three cases in which the histologic findings were false negative. (AM. J. OBSTET. GYNECOL. 139:148, 1981.)

THE CONCEPT OF diagnostic needle aspiration was born more than 50 years ago. It was first described by Guthrie,<sup>1</sup> in 1921, and later by Stewart,<sup>2</sup> in 1933, and by Martin and Ellis,<sup>3</sup> in 1934. The early techniques used larger needles, usually 18 gauge; our current technique would make the use of this larger sized needle somewhat hazardous. The technique is to be clearly differentiated from a needle biopsy in which a core of tissue is obtained and the tissue is processed for histologic section. In fine-needle aspiration cytology, the specimen is prepared and interpreted according to established cytologic rather than histologic criteria. The current technique and instrumentation stem predominantly from the works of Franzén and Zajicek in Scandinavia. They have acquired extensive experience with transrectal prostatic aspirations,<sup>4</sup> aspirations of lymph nodes in the neck,<sup>5</sup> and aspiration of solid and cystic breast lesions.<sup>6</sup> The first report on the application of fine-needle aspiration cytology to the management of gynecologic oncology patients was made in 1979 by

Sevin and associates,<sup>7</sup> who reported a 96.4% accuracy in differentiating between malignant and benign conditions. Additionally, in their institution with its strong emphasis on the subspecialty of gynecologic pathology and cytology, they were able to predict accurately the histologic diagnosis by means of the fine-needle cytology in 97.9% of cases studied.

The data that we now report were accumulated over a 2-year period as an integral part of the care of gynecologic oncology patients.

## Material and methods

Shown in Fig. 1 is the equipment that we use, depending upon the site that we wish to evaluate. We consider that the 20 ml syringe holder-aspirating device is essential since it allows increased suction to be placed on the syringe with one hand while the other hand is localizing or fixing the mass to be aspirated. Also pictured is the Franzén needle guide which we use for some transvaginal aspirations. It is especially useful, however, for transrectal aspirations to help guide the needle to the proper spot. The three needle sizes which we consider to be essential are also shown in the photograph. They are a 20 cm, 22-gauge disposable Chiba needle for use through the Franzén needle guide; an 8.9 cm, standard 22-gauge spinal needle; and a 3.8 cm, standard 22-gauge needle.

Most sites that we aspirated were localized by palpation. Occasionally, we employed ultrasonography. The fluoroscope was also used to guide us toward an inter-

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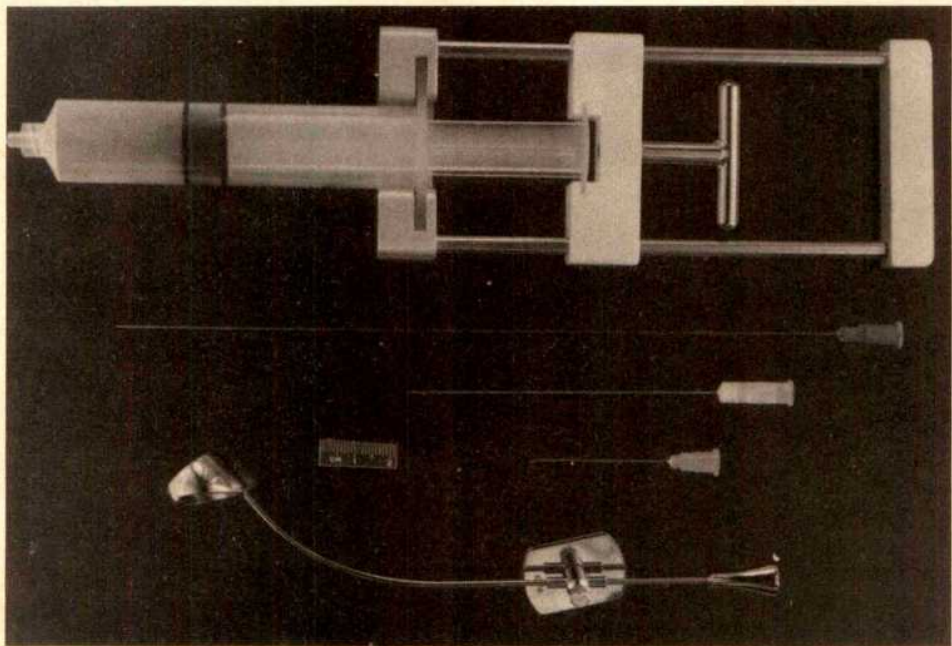


Fig. 1. The instruments used for fine-needle aspiration cytology.

nal site, such as an opacified para-aortic lymph node.

Superficial sites, such as lymph nodes, were most frequently sampled by means of the 3.8 cm, 22-gauge needle. Palpable abdominal tumors and most masses palpable at the vaginal apex were sampled with the 8.9 cm, 22-gauge spinal needle. With the use of the Franzén needle guide and the 20 cm, 22-gauge Chiba needle, all other areas of the true pelvis could be reached either transvaginally or via a transrectal approach. The usual situation was one in which no material was visible within the syringe, with the aspirated material being totally contained within the needle. Under these circumstances, the needle was emptied onto a slide, the material was spread with a second slide, and the two slides were immediately sprayed with fixative. The material was then stained by the standard Papanicolaou technique. If a large amount of aspirated material was collected, it was placed in a rubber-stoppered tube with a few drops of heparin and sent to the cytology laboratory. The fluid was centrifuged, any sediment was processed for cell block, and the supernatant was used for cytologic study.

The diagnoses were confirmed either histologically at the time that the fine-needle aspiration was performed (46 cases) or within a brief interval after exploratory laparotomy (22 cases), or by the obvious clinical situation (22 cases). This last group of cases consisted of three subgroups. The first group comprised patients in whom more than one site was sam-

Table I. Location of fine-needle aspiration samples

| Sites                                       | No. |
|---------------------------------------------|-----|
| <i>Lymph nodes:</i>                         | 13  |
| Para/aortic                                 | 1   |
| Supraclavicular                             | 3   |
| Inguinal                                    | 7   |
| Common iliac                                | 1   |
| Obturator                                   | 1   |
| Cul-de-sac (random sampling and/or nodules) | 37  |
| Peritoneal fluid                            | 4   |
| Intra-abdominal masses                      | 2   |
| Pelvic mass                                 | 6   |
| Parametrium                                 | 17  |
| Uterosacral ligament                        | 2   |
| Bladder                                     | 1   |
| Anterior rectal wall                        | 1   |
| Posterior sacral mass                       | 1   |
| Liver                                       | 1   |
| Vaginal nodules                             | 4   |

Table II. Distribution of fine-needle aspirations by diagnosis

| Clinical diagnosis                   | Total | Un-treated | After therapy |
|--------------------------------------|-------|------------|---------------|
| Carcinoma of ovary                   | 47    | 2          | 45            |
| Carcinoma of cervix                  | 16    |            | 16            |
| Carcinoma of endometrium             | 7     |            | 7             |
| Carcinoma of colon                   | 5     |            | 5             |
| Carcinoma of vulva/vagina            | 5     | 1          | 4             |
| Carcinoma of fallopian tube          | 1     |            | 1             |
| Pelvic mass/right ovarian malignancy | 6     | 6          | 0             |
| Carcinoma of breast                  | 3     |            | 3             |
| Total                                | 90    | 9          | 81            |



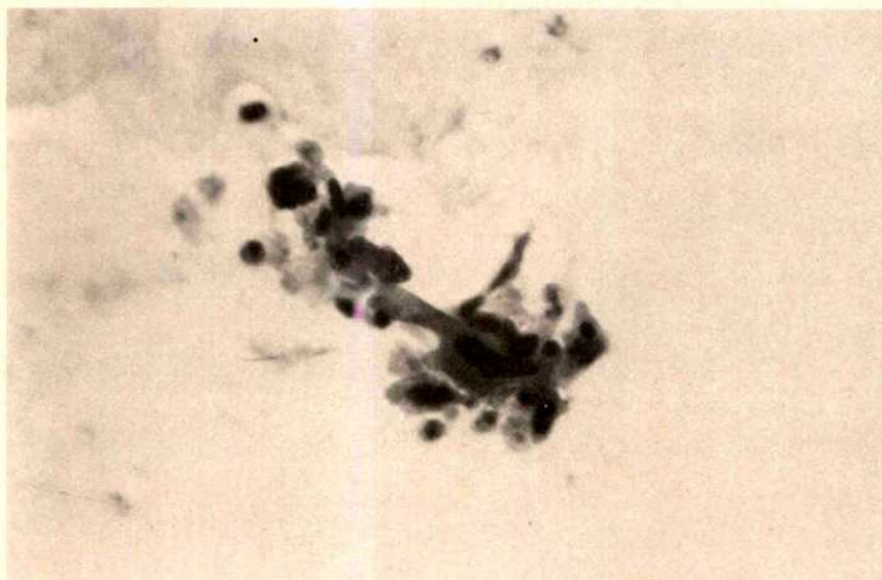


**Fig. 2.** Case 1. Sheet of tumor cells with keratinized cytoplasm, irregular nuclear borders, and "india ink" nuclei. Diagnosis: Well-differentiated keratinized squamous cell carcinoma. ( $\times 400$ .)

pled with the fine needle, but not all were biopsied. This occurred when an aspiration exactly resembled a previous sample that had biopsy confirmation. In the second group were patients who had negative findings by random follow-up sampling of the pelvis and either refused a second-look procedure or had had one 6 months to 1 year previously. These patients have now been observed a minimum of 8 months. The third group was made up of patients with extensive cancers in whom the biopsy was considered to be superfluous and/or risky.

### Results

Table I shows the location of the fine-needle aspiration samples that we are reporting. Table II shows the distribution of the cases by diagnoses, and also that the fine-needle technique has been employed predominantly for following patients after therapy. The aspirations fall into three categories: those that were performed for initial diagnosis; those that were done after therapy in which specific findings, such as a nodule, were evaluated; and those performed after therapy in which the pelvis was randomly sampled without specific clinical findings. Three case reports are presented in order to demonstrate these categories.

### Case reports

**Case 1.** P. J., a 27-year-old white woman, presented with an ulcerative, keratinizing squamous cell carcinoma of the cervix. A preoperative intravenous pyelogram was normal. However, at the time of examination

under anesthesia, a 2 cm pelvic sidewall nodule was appreciated. A transvaginal fine-needle aspiration of the right pelvic sidewall nodule was done, and the cytologic examination revealed keratinizing squamous cell carcinoma (Fig. 2). At exploration, a positive 2 cm right obturator node was confirmed, as well as the presence of positive para-aortic nodes. This is an example of use of the technique for primary diagnosis.

**Case 2.** E. L., a 55-year-old woman, presented with a Stage IIA nonkeratinizing squamous cell carcinoma of the cervix. At exploration, she was found to have a positive para-aortic node. Sixteen months after pelvic and para-aortic node radiation, the patient developed pain in a right sciatic distribution, and an intravenous pyelogram showed a partial ureteral obstruction at the level of the pelvic brim. A lymphangiogram showed a highly suspicious right common iliac lymph node. Under fluoroscopic guidance, a fine-needle aspiration was performed on the common iliac lymph node, and this revealed nonkeratinized squamous cell carcinoma (Fig. 3). This is an example of an evaluation of a specific finding after therapy.

**Case 3.** J. T., a 40-year-old white woman, presented with a Stage III, poorly differentiated adenocarcinoma of the ovary. Fourteen months after surgical debulking and multiple-drug chemotherapy, the patient was totally without clinical evidence of disease. Coincidental to the performance of cystoscopic examination for hematuria, a fine needle was passed into the cul-de-sac, and the pelvis was randomly sampled. Recurrent adenocarcinoma was diagnosed on fine-needle aspiration and later confirmed by exploratory operation (Fig. 4). This is an example of random pelvic sampling at fol-



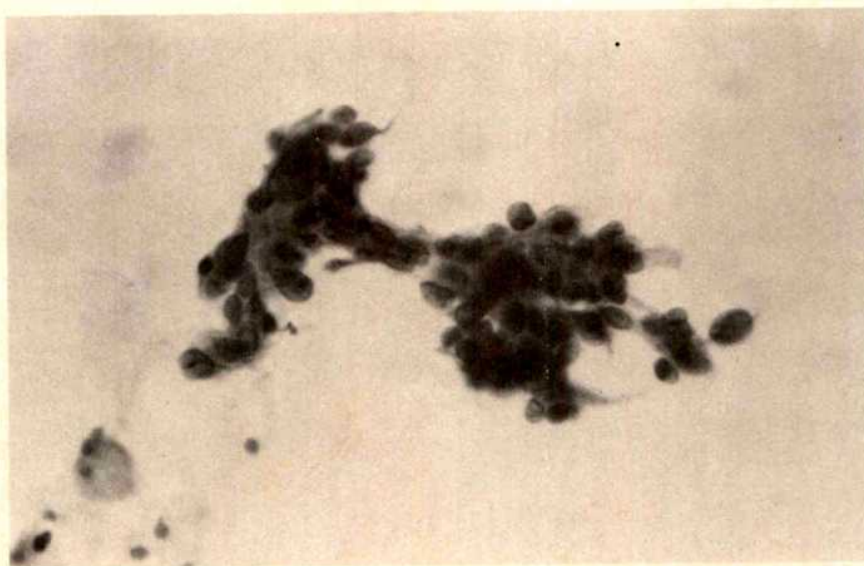


**Fig. 3.** Case 2. Monolayer sheet of malignant cells exhibiting anisokaryosis and hyperchromasia. No cytoplasmic differentiating features are apparent. Diagnosis: Poorly differentiated squamous cell carcinoma. ( $\times 400$ .)

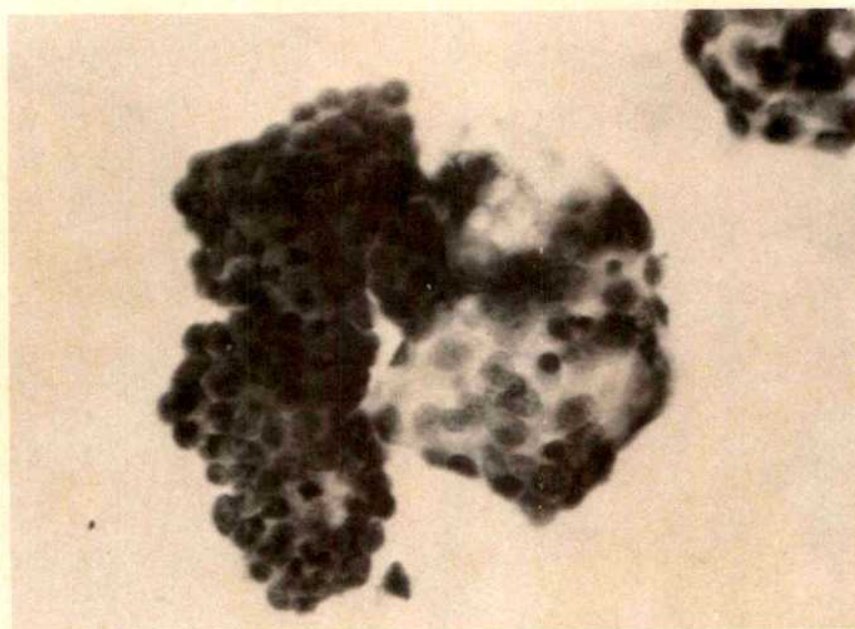


**Fig. 4.** Case 3. Three-dimensional cluster of tumor cells in a papillary configuration. Diagnosis: Poorly differentiated adenocarcinoma. ( $\times 400$ .)

low-up without evidence of a specific recurrence.

Table III summarizes the results. The fine-needle aspiration diagnosis was recorded as positive in 48 cases and benign in 37 cases, and the aspiration was insufficient in five cases. All diagnostic discrepancies occurred in the group with biopsy correlation. This is not surprising when the makeup of the group which did not undergo biopsy is considered. As noted in the table,

there was one false positive aspiration which was an aspiration of an enlarged inguinal lymph node in a patient with extensive extramammary Paget's disease. The cytologic examination revealed several small sheets of cells with enlarged nucleoli. Removal of the lymph node showed a sinus histiocytosis. There were two false negative aspirations, both of which were fairly early in our experience with the technique and both of which



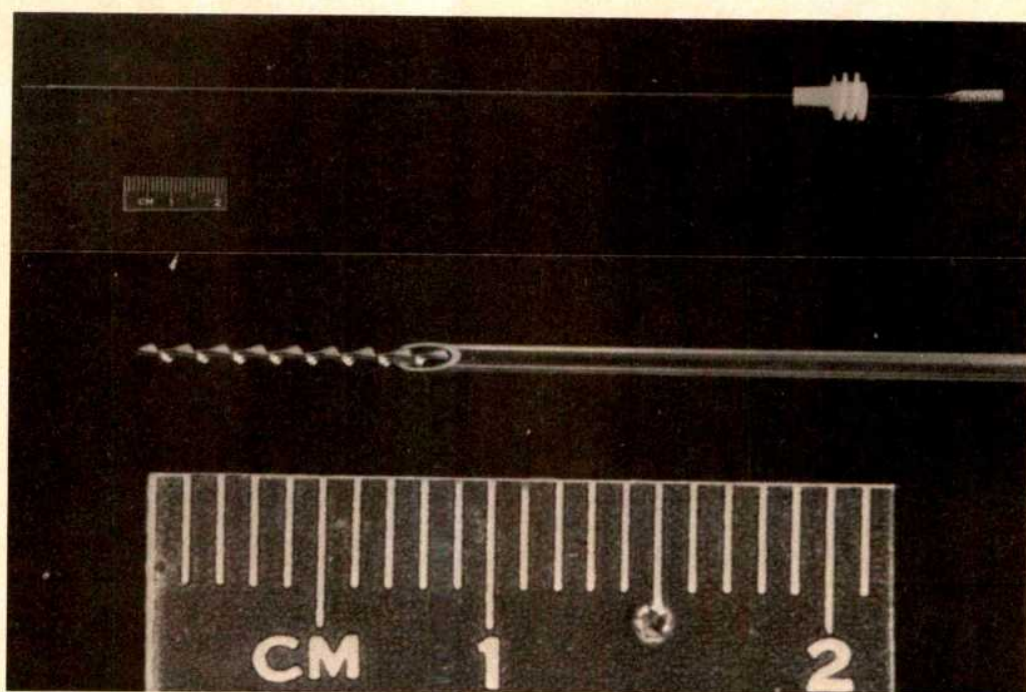


Fig. 5. Photograph of the Nordenström screw (screw, 0.55 mm; cannula, 0.8 by 172 mm).

Table III. Results of fine-needle aspiration

| <i>Fine-needle aspiration diagnosis</i> | <i>Total</i> | <i>False negative diagnosis</i> | <i>False positive diagnosis</i> |
|-----------------------------------------|--------------|---------------------------------|---------------------------------|
| Malignant                               | 48*          |                                 | 1                               |
| Benign                                  | 37           | 2                               |                                 |
| Insufficient                            | 5            |                                 |                                 |
| Total                                   | 90           | 2                               | 1                               |

\*Three patients had false negative histologic diagnosis.

occurred in large masses. The aspiration had not begun close enough to the surface of the mass, and we obtained primarily necrotic debris from the central portions of the mass. Five aspirations were insufficient since the amount of cellular material on the slide was too sparse for evaluation. It should be noted that there were three patients in whom the fine-needle aspiration showed a malignancy although the initial biopsies were all negative. These individuals had pelvic masses just above the vaginal apex. Biopsy specimens were initially taken of the visible vaginal portion, and in these three instances only necrotic and inflammatory tissue was present. This demonstrated one of the advantages of the fine-needle aspiration technique, namely, that the needle passes through the entire mass, thereby sampling multiple areas. Additional biopsies at deeper sites were performed in all these patients, and the presence of malignancy was confirmed.

### Comment

At our institution, fine-needle aspiration cytology is an integral part of the practice of gynecologic oncology. In fact, we are significantly dependent upon this technique. The ease and rapidity with which otherwise diagnostic dilemmas can be solved are impressive. Frequently, a diagnosis is made that would otherwise require laparotomy.

In the process of staging a patient who has a disease such as cervical cancer, the fine needle has obvious adjunctive applications. Since random sampling is not used at our institution during initial diagnostic phases, the fine-needle results would not be standing alone as a contribution to determining the patient's stage. However, frequently it can be applied to add support to clinical findings (as in Case 1), or to influence one's decisions when the clinical findings are equivocal in nature. The use and approval of fine-needle aspiration in the staging process in general will have to be determined in the future as the availability of the technique expands.

We believe that the technique is an invaluable element in the practice of gynecologic oncology. However, it must be emphasized that an interested and skilled cytology department is essential. An additional point to make is that, contrary to the studies from the University of Miami,<sup>8</sup> the cytopathologic interpretation was made by two of us who are general pathologists and not



subspecialists in gynecologic pathology. We think that this is an extremely important point, in that a general pathologist well versed in gynecologic pathology as well as cytology can provide reliable diagnoses. Additionally, the point has been made by the Miami group that diagnoses such as a "positive" or "negative" are "absolutely meaningless,"<sup>9</sup> and that a diagnosis with the use of histologic terminology is essential. We use histologic terminology when possible. However, the interpretation of the slide as positive or negative for malignancy provides useful information at that level even without the use of a histologic description.

We have been impressed by the ease with which the technique can be used, and by the high degree of acceptability on the part of the patient. Seventy-three percent of the fine-needle aspirations have been performed without the use of anesthesia. The aspirations are handled in priority fashion by the cytopathology laboratory, and the results are available routinely within a few hours.

We have had no complications that resulted from the use of the fine-needle aspiration technique. For many of the intra-abdominal aspirations, the needle must pass through loops of bowel and may occasionally strike a vessel, but, in our experience, this has not caused any complications, because of the thinness of the needle. A very large worldwide experience in the evaluation of nongynecologic neoplasms supports the absence of complications. Not enough time has elapsed to determine whether aspiration of the tumors has a detrimental effect on prognosis. However, a report by

Berg and Robbins<sup>10</sup> evaluates 1,412 patients who underwent diagnostic aspirations of breast lesions, and a 15-year follow-up demonstrated no effect on prognosis. Nonetheless, we are still not prepared to use the fine needle to evaluate primary ovarian masses, because of the potential risks of peritoneal contamination. Kjellgren and associates<sup>11</sup> discuss this procedure but make no statement on the safety of the technique in terms of contamination of the peritoneal cavity with viable tumor cells.

Currently, we are comparing the fine-needle aspiration technique with the use of the Nordenström screw (Fig. 5),<sup>12</sup> since this particular fine-needle diagnostic technique may be simpler and more applicable in the evaluation of certain types of masses.

It is worth mentioning that one should not become too rigid in the matter of fine-needle aspiration cytology and apply it just to the evaluation of masses or to random sampling. The thinness of the needle is the critical factor which permits the obtaining of fluid from the abdomen or the chest with great safety and minimal discomfort to the patient. This is an appropriate extension of the technique.

As one views the use of fine-needle aspiration, the advantages are clearly simplicity of technique, markedly reduced costs and hospitalization time, and a reduced risk of trauma associated with diagnostic procedures. The technique has proved to be accurate and its use is readily acceptable to the patient. The only major drawback to the technique is its dependence upon good-quality cytologic evaluation.

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# The significance of atypical vessels and neovascularization in cervical neoplasia

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The relationship between atypical vessels seen colposcopically and dysplasia, carcinoma in situ (CIS), microinvasion, and frank invasion was studied quantitatively. No atypical vessels were found with dysplasia, but 2.8% of patients with CIS had atypical vessels. Half of the patients with microinvasion and all of the patients with frank invasion, in whom the entire zone of transformation was viewed, had atypical vessels. Eighty-two percent of the patients with atypical vessels had invasion. The conclusions are: (1) Atypical vessels are not present with dysplasia and rarely present with CIS. (2) Atypical vessels may be associated with microinvasion, but are required for frank invasion to occur. (3) Because atypical vessels are usually associated with invasion, which can be in or near the field of atypical vessels, diagnostic cone biopsy should be performed if atypical vessels are seen and colposcopic biopsies do not show frank invasion. (4) Microinvasion without atypical vessels may be a localized disease. (AM. J. OBSTET. GYNECOL. 139:154, 1981.)

THE MICROCIRCULATION provides important clinical clues in colposcopy for the evaluation of cervical neoplasia<sup>1, 2</sup> and is also of biologic importance in understanding carcinogenesis.<sup>3-6</sup>

The normal, terminal microcirculation of the cervix is characterized by fine, subepithelial, reticular vessels. Epithelium with increased metabolic demand, due to active metaplasia, inflammation, repair, or intraepithelial neoplasia, is characterized by punctation and mosaicism which represent a "remodeling of the pre-existing (vascular) network."<sup>6</sup> Vessels that are nourishing intraepithelial neoplasia are distinguished by an increased coarseness, irregularity, and intercapillary distance. Atypical vessels represent new vessels, possibly humorally induced by a tumor angiogenic factor.<sup>3</sup> At their earliest stage, atypical vessels bridge the interface between in situ and invasive cancer and have clinical and biologic significance which is not completely understood.

Atypical vessels are qualitatively associated with in-

vasive cancer. However, the quantitative data support both sides of the debate on whether atypical angiogenesis precedes or follows invasion. Uncertainty about the significance of atypical vessels is exemplified by the varying degrees of diagnostic thoroughness used in evaluating patients in whom they are found. An important question is whether diagnostic conization should be performed in patients with atypical vessels if invasion is not found on colposcopically directed biopsies and endocervical curettage (ECC).

Three measures reflect the quantitative association of atypical vessels with invasive cancer: (1) the frequency of atypical vessels in patients with intraepithelial neoplasia; (2) the frequency of atypical vessels in patients with invasive cancer; and (3) the frequency of invasive cancer in patients with atypical vessels.

The purpose of this study was to provide more data on the three above-listed measures in order to help clarify clinical management and to lend circumstantial evidence to the query of whether atypical angiogenesis is a prerequisite for or the result of invasion.

## Material and methods

Patients who were found to have atypical vessels on colposcopy at the Downstate Medical Center during the years 1976-1978 are the principal focus of this study. Patients with macroscopically visible invasive cancers were excluded from the study, since such lesions were usually biopsied directly.

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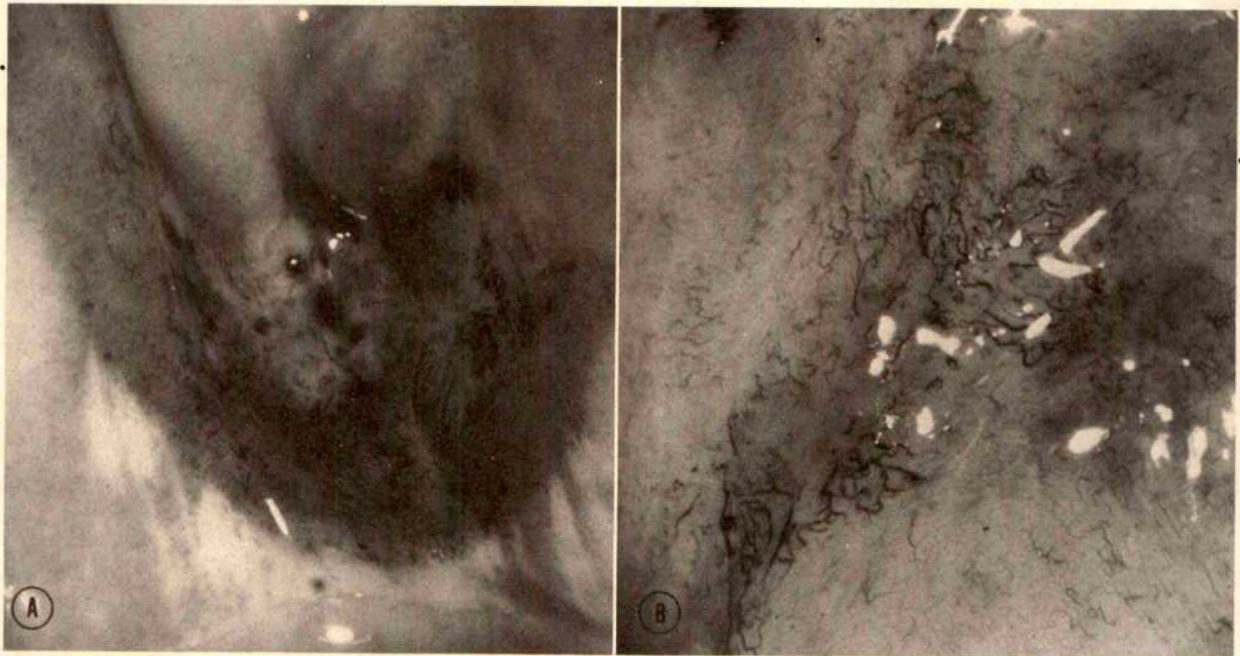


Fig. 1. Bizarre vessels, with cervicitis (A) and in the vagina after radiation therapy (B).

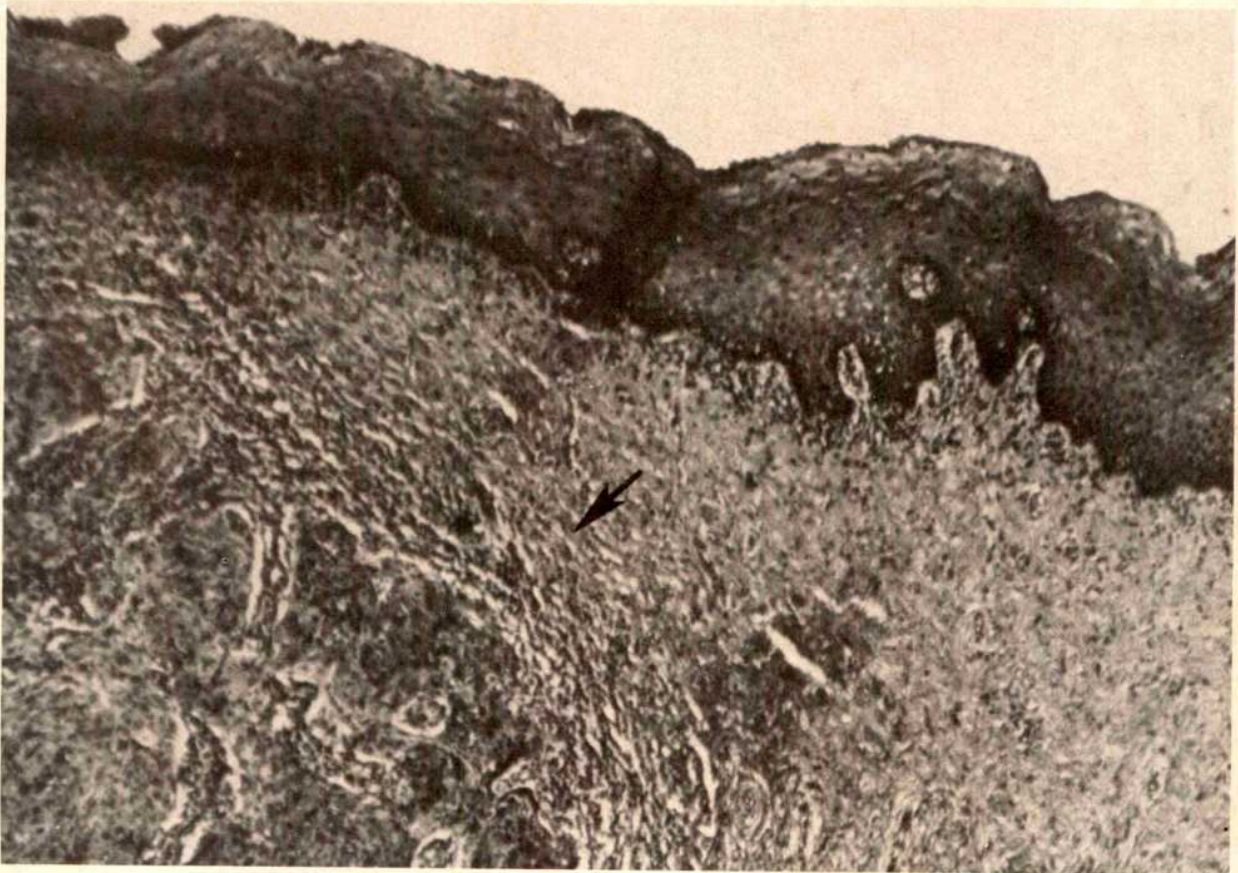


Fig. 2. Distal endocervix showing invasive cancer (arrow) infiltrating underneath epithelium from more proximal endocervix.



**Table I.** Frequency of atypical vessels in patients with cervical neoplasia

| Histology                  | Patients examined | Atypical vessels |         | Frequency of atypical vessels (%) |
|----------------------------|-------------------|------------------|---------|-----------------------------------|
|                            |                   | Absent           | Present |                                   |
| Moderate dysplasia or less | 1,481             | 1,481            | 0       | 0                                 |
| Severe dysplasia           | 487               | 487              | 0       | 0                                 |
| Carcinoma in situ          | 390               | 379              | 11      | 2.8                               |
| Microinvasion              | 8                 | 4                | 4       | 50                                |
| Frank invasion             | 49                | 4                | 45      | 92                                |
| Total                      | 2,415             | 2,355            | 60      | 2.5                               |



**Fig. 3.** Colpophotograph of aceto-white lesion containing coarse punctuation and four atypical vessels (encircled).

Microinvasion was considered to be invasion that was 3 mm or less below the basement membrane without vascular involvement.

Kolstad and Staff's definition of atypical vessels, magnificently illustrated by their colpophotographs, was used.<sup>1</sup> Atypical vessels are two to ten times wider than normal capillaries<sup>7</sup> and are irregular in width, shape, and course. They have a horizontal component and abruptly appear and disappear. They are separated from each other, often widely, and form no regular pattern, although occasionally they may simulate a deranged network. Atypical vessels can be confused with "bizarre" vessels which bear some resemblance to

them but do not fulfill all of the criteria for them. Bizarre vessels may be prominent and tortuous, but irregularities are less abrupt and intercapillary distance is less than that with atypical vessels. Bizarre vessels are not accompanied by other colposcopic indications of cancer, such as a focal lesion with darkened hues and irregular contours. Bizarre vessels are infrequently seen after trauma, such as infection, erosion, previous biopsy, cautery, cryosurgery, or laser treatment, but more frequently after radiation (Fig. 1).

All but one patient with atypical vessels, and no invasion on colposcopically directed biopsies or endocervical curettage, underwent a cone biopsy and/or hysterectomy. Even though that one patient was lost to follow-up after colposcopy, she is included in the group of patients with atypical vessels and no invasion, since we were testing the hypothesis that atypical vessels are always associated with invasive cancer and vice versa.

**Results**

During the 3-year study period, 2,415 patients were examined for cervical neoplasia (Table I). Of the 60 patients who had atypical vessels, all had either in situ or invasive cancer. No patient with dysplasia had atypical vessels.

**Frequency of atypical vessels in patients with carcinoma in situ.** Three hundred and ninety patients had carcinoma in situ (CIS), and 11 (2.8%) of these had atypical vessels. In seven of the 11 (64%) the cytologic features were suggestive of invasion.

**Frequency of atypical vessels in patients with invasive cancer.** Four patients (50%) with microinvasion had atypical vessels, and the mean depth of invasion of the cancer was 0.6 mm. The mean depth of invasion was 1.75 mm in the four patients in whom atypical vessels were not seen. The entire zone of transformation was seen in the four patients who did not have atypical vessels.

Forty-five patients (92%) with occult frank invasion had atypical vessels. In the four patients without atypical vessels, the view was not inclusive of the entire zone of transformation, so that, possibly, atypical vessels, were present but not seen.



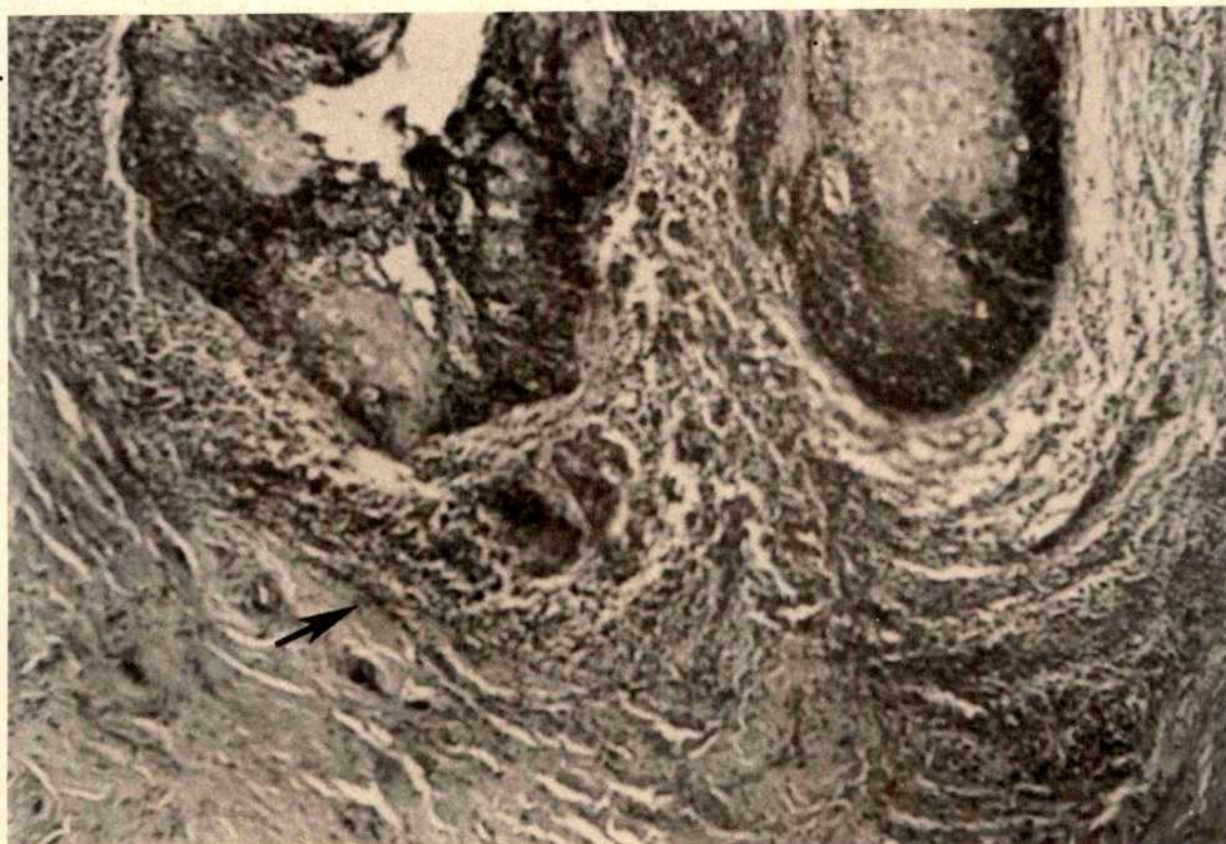


Fig. 4. Microinvasion (arrow), 0.1 mm, found in endocervix adjacent to aceto-white lesion seen in Fig. 3.

Table II. Frequency of atypical vessels reported by different authors

| Author                           | Frequency of atypical vessels |                   |               |                         |
|----------------------------------|-------------------------------|-------------------|---------------|-------------------------|
|                                  | Severe dysplasia              | Carcinoma in situ | Microinvasion | Frank (occult) invasion |
| Sillman, Boyce and Fruchter      | 0% (0/487)                    | 2.8% (11/390)     | 50% (4/8)     | 92% (45/49)             |
| Johannisson et al. <sup>14</sup> | 0% (0/23)                     | 2% (1/47)         | "Often"/18*   |                         |
| Ogawa and Maki <sup>13</sup>     | 0% (0/40)                     | 4% (3/81)         | 40% (14/35)   |                         |
| Noda <sup>8</sup>                | 0% (0/69)                     | 3% (3/102)        | 67% (24/36)   | 3-5 mm; <67%*           |
| Sugimori et al. <sup>9</sup>     | 3% (1/37)                     | 6% (4/66)         | 33% (14/43)   |                         |
| Van Meir et al. <sup>15</sup>    |                               |                   | 0% (0/9)      | 3-5 mm; 73% (8/11)      |
| Kuramoto et al. <sup>12</sup>    |                               |                   | 61% (36/59)   |                         |
| Sasaki et al. <sup>11</sup>      |                               |                   | 30%*          |                         |
| Tovell et al. <sup>10</sup>      |                               | 16% (6/37)*       |               | 88% (23/26)*            |
| Range                            | 0-3%                          | 2-6%              | 33-67%        | 73-92%                  |
| Mean                             | 0.15% (1/656)                 | 3.2% (22/686)     | 51% (92/181)  | 88% (53/60)             |

\*Not included in range and mean figures because definitive data were not available.

**Frequency of invasive cancer in patients with atypical vessels.** Of the 60 patients with atypical vessels, 49 (82%) had invasion. In four patients, the field of atypical vessels was biopsied and only CIS was found. However, additional tissue revealed invasion nearby in the endocervix. Because the findings in the latter four

cases were particularly instructive, they are presented here in brief.

J. D., 40 years old, had a Papanicolaou smear of severe dysplasia. The colposcopic view was inclusive of the entire zone of transformation, and atypical vessels



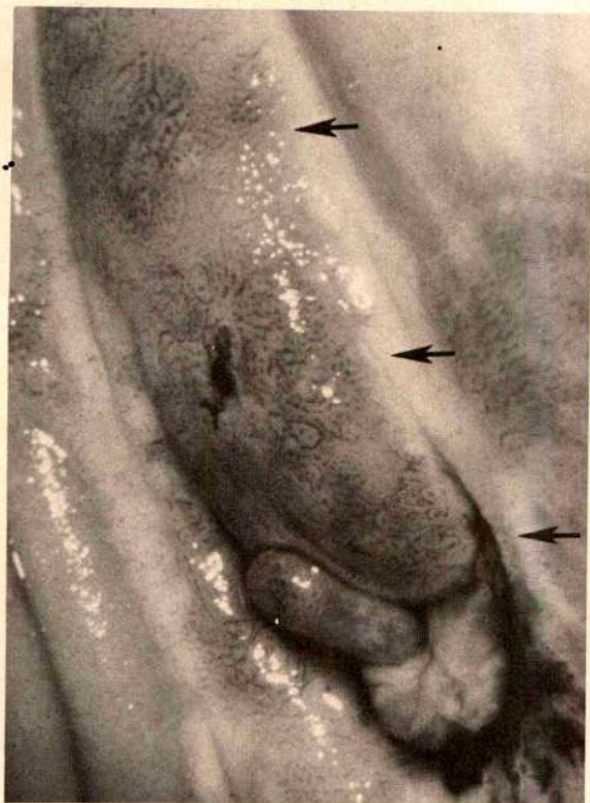


**Fig. 5.** A few, early, atypical vessels on anterior exocervix (opening of mosaics and slight "tadpoling," arrows), heralding invasion in anterior endocervical canal.

were seen on the exocervix. The colposcopic impression was microinvasion. Biopsy specimens from the area revealed CIS. The ECC was negative. A hysterectomy was performed for CIS, and the uterus contained an area of frank invasion in the endocervix that was partly covered by noninvasive epithelium (Fig. 2).

V. K., 57 years old, had cells suggestive of invasion on a Papanicolaou smear. The colposcopic view was not inclusive of the entire zone of transformation. Atypical vessels were seen. Biopsy specimens from an endocervical lesion and ECC showed CIS. The diagnostic cone showed frank invasion in the endocervix.

E. Y., 41 years old, had a Papanicolaou smear compatible with CIS. She had a focal aceto-white lesion with four atypical vessels (Fig. 3), which was excised and showed CIS. At hysterectomy, 0.1 mm of microinvasion was found in the adjacent endocervix (Fig. 4).

D. M., 43 years old, had a Papanicolaou smear of CIS. She was 14 weeks' pregnant. A few, early, atypical vessels were seen on the exocervix (Fig. 5). Biopsy of this area revealed CIS. Visually directed biopsy of the distal endocervical canal was performed with a curet because of the atypical vessels on the exocervix and slight contour irregularities of the endocervix, and frank invasion was found. The radical hysterectomy specimen contained 7 mm of invasion.

### Comment

Table II shows the frequency of atypical vessels reported in this study and by other authors. Five investigators found atypical vessels in 0.15% of patients with severe dysplasia. The frequency for CIS was 3.2%.

Atypical vessels were seen in 51% of patients with microinvasion. Noda,<sup>8</sup> Sugimori,<sup>9</sup> and Tovell<sup>10</sup> and their associates defined microinvasion as invasion up to 3 mm. Sasaki,<sup>11</sup> Kuramoto,<sup>12</sup> Ogawa,<sup>13</sup> and Johannisson<sup>14</sup> and their associates did not define the depth of microinvasion. The data from the Downstate Medical Center did not suggest a correlation between atypical vessels and the depth of invasion when the invasion was less than 3 mm. All patients whom we have seen with invasion greater than 3 mm, and in whom the entire zone of transformation was viewed, had atypical vessels. Noda found atypical vessels in 67% of patients with up to 3 mm of invasion, and atypical vessels less often with 3 to 5 mm of invasion because of the "greater distance of the vessels from the surface." Van Meir and associates<sup>15</sup> saw no atypical vessels in patients with 1 to 3 mm of invasion, but the entire zone of transformation was not viewed in most of their patients. They saw atypical vessels in eight of 11 (73%) patients with 3 mm to 5 mm of invasion.

Although Table II reveals some quantitative differences, there is surprising agreement on the frequency of atypical vessels with various stages of cervical neoplasia, despite the subjectivity and experience involved in the interpretation of atypical vessels. This agreement is supported by the work of Koller<sup>7</sup> and his review of the literature prior to 1962. The variations in terminology and lack of quantitative data make quantitative assessment conjectural prior to the middle 1960s.

A most important question is whether atypical vessels are a predecessor and prerequisite for invasion or whether atypical vessels result from invasion. The finding of Staff and Mattingly<sup>6</sup> of progression to microinvasion within 6 months in six patients with atypical vessels and CIS, and our findings of invasive cytologic features in 64% of our patients with atypical vessels and CIS, suggest that such patients have an imminently invasive neoplasm. However, the authors listed in Table II found that 49% of the patients with microinvasion did not have atypical vessels. This favors the belief that neovascularization is not necessary for microinvasion, and is consistent with the experimental evidence cited by Folkman and associates<sup>3, 4, 16</sup> which indicates that malignant tumors up to 2 to 3 mm in diameter can be nutritionally sustained by diffusion alone. Why a few patients with CIS have atypical vessels and why many with microinvasion do not is not clear. Whether atypical vessels require only that an angiogenic factor be secreted is not yet known. Evidence exists that there are other prerequisites, such as the



location of capillaries within 3 mm of the secreting neoplastic cells,<sup>16</sup> and neoplastic microtrauma to the capillary endothelium.<sup>17</sup> The latter phenomena may occur by chance in approximately 50% of patients with microinvasion. With frank invasion, not only are these phenomena more likely to occur, but neovascularization is necessary to sustain the larger volume of tumor.

Several clinical implications can be drawn. Any of the following criteria are currently accepted as an indication for diagnostic cone biopsy. (1) The colposcopic view is not inclusive of the entire zone of transformation. (2) Histologic features are more than one step below the cytologic findings. (When the cytologic findings suggest invasion, frank invasion must be found on colposcopic biopsy for a definitive diagnosis.) (3) The ECC specimen contains malignant epithelium without a diagnosis of frank invasion. (A diagnostic conization is sometimes indicated if dysplasia is found on ECC.) (4) The initial biopsy specimens reveal microinvasion.

Our finding that 82% of patients with atypical vessels had invasion, and that invasion may be near (possibly secreting an angiogenic factor) but not in the field of

atypical vessels, led us to conclude that the presence of atypical vessels without frank invasion is a fifth indication for diagnostic cone biopsy. In several patients, atypical vessels were the earliest and only clue to invasion.

Conservative therapies for CIS that do not yield additional histologic findings (i.e., cryosurgery, cautery, laser therapy, immunotherapy, topical 5-fluorouracil) are dangerous in the presence of atypical vessels.

In like fashion, microinvasion with atypical vessels may be more malignant than its histologic equivalent without atypical vessels. Averette and associates<sup>18</sup> made a strong case for nonmetastasis of microinvasion of 1 mm or less. To date, this is the only degree of invasion that has been proved to be "100%" safely treatable by nonradical means. It is possible that microinvasion of 1 mm to 3 mm without atypical vessels also carries no risk of metastasis and could be treated nonradically.<sup>19</sup> Microinvasion of more than 1 mm with atypical vessels may be treated more surely with radical surgical procedures or radiation. This is highly speculative and yet to be proved.

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# Effect of cyproheptadine on chlorpromazine stimulation of prolactin in women

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In five normal women, the expected chlorpromazine-induced rise in serum prolactin was inhibited by pretreatment with a serotonin antagonist, cyproheptadine. This indicated that the serotonergic system has an excitatory effect on the release of prolactin. When similar studies were done in nine women with hyperprolactinemia and galactorrhea, the administration of chlorpromazine did not consistently produce a rise in serum prolactin, and pretreatment with cyproheptadine did not have a predictable effect. In most cases, this chlorpromazine-induced reduction in the prolactin inhibitory factor could not further increase the secretion of prolactin, so that there was no rise to inhibit.

(AM. J. OBSTET. GYNECOL. 139:160, 1981.)

THE ADMINISTRATION of serotonin precursors has been shown to stimulate the secretion of human prolactin.<sup>1, 2</sup> Such precursors also stimulate thyrotropin. This implies that serotonin may act as a hypothalamic neurotransmitter in the production of thyrotropin-releasing factor (TRF), which also acts as a prolactin-releasing factor.<sup>3</sup>

The antiserotonin agent cyproheptadine<sup>4</sup> has been shown to blunt the stimulatory effect on prolactin of 5-hydroxytryptophan, a serotonin precursor,<sup>5</sup> and either to cause a lowering of<sup>6</sup> or to have no effect on<sup>5</sup> the levels of prolactin in women with hyperprolactinemia.

Chlorpromazine induces a rise in the levels of prolactin in normal women,<sup>7</sup> by acting on the hypothalamus to reduce the release of prolactin inhibitory factor (PIF), thereby freeing the pituitary to secrete an increased amount of prolactin.<sup>8</sup>

This study was undertaken to determine whether inhibition of the serotonergic system would alter the response of women (both normal women and those with hyperprolactinemia) to the administration of chlorpromazine, and to give additional evidence that

serotonin is a neurotransmitter involved in the control of the secretion of prolactin.

## Material and methods

The five study participants who were designated as normal controls were paid volunteers who were not pregnant, had regular menses, had no known disease, had taken no medication for 2 weeks, and did not have bronchial asthma. In addition, all screening levels of serum prolactin had been normal.

The study participants who had hyperprolactinemia and galactorrhea are described in Table I. These women likewise were of reproductive age but not pregnant, had taken no medication for 2 weeks, and did not have bronchial asthma. Polytomography of the sella turcica and visual-field examinations were performed in order to investigate the possibility that a pituitary adenoma was present. Although there were x-ray changes compatible with a microadenoma in seven of nine women, in no case was a macroadenoma present.

Informed consent was obtained from all study participants, and approval was granted by the institutional human research committee. The project was conducted in the Clinical Research Center. After the women had fasted overnight from 11 PM, "heparin lock" needles were inserted between 8 and 9 AM, and 5-ml samples of blood were obtained as controls. An intramuscular injection of chlorpromazine, 25 mg, was given, and subsequent 5-ml samples of blood were obtained ½, 1, 1½, 2, and 3 hours later. When pretreatment with a serotonin inhibitor was administered, oral

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**Table I.** Characteristics of study participants with hyperprolactinemia and galactorrhea

| Case No. | Serum prolactin (ng/ml) | Amenorrhea | Polytomography of sella turcica                                                                 | Follow up                                                                                                                                        |
|----------|-------------------------|------------|-------------------------------------------------------------------------------------------------|--------------------------------------------------------------------------------------------------------------------------------------------------|
| 1.       | 91                      | Yes        | Slight erosion of the anterior sella floor and marked erosion of the dorsum sellae              | Developed spontaneous menses                                                                                                                     |
| 2.       | 83                      | Yes        | Initially normal but 7 months after studies repeat polytomography showed erosion of sella floor | Transphenoidal resection of pituitary microadenoma with initial improvement but later return of hyperprolactinemia, galactorrhea, and amenorrhea |
| 3.       | 47                      | Yes        | Erosion of the inferior posterior wall of the sella                                             | Transphenoidal resection of pituitary microadenoma followed by pregnancy                                                                         |
| 4.       | 257                     | Yes        | Minimal erosion of the anterior floor of the sella                                              | Transphenoidal resection of microadenoma                                                                                                         |
| 5.       | 116                     | Yes        | Ill-defined sella floor                                                                         | Transphenoidal resection of pituitary microadenoma followed by pregnancy                                                                         |
| 6.       | 163                     | Yes        | Very thin lamina dura with questionable focal posterior inferior sella floor erosion            | Became pregnant without operation or medication                                                                                                  |
| 7.       | 116                     | Yes        | Minimal erosion of proximal posterior floor of sella                                            | Seven months later developed spontaneous menses and 11 months later became pregnant                                                              |
| 8.       | 148                     | No         | Normal                                                                                          |                                                                                                                                                  |
| 9.       | 141                     | No         | Normal                                                                                          |                                                                                                                                                  |

**Table II.** Serum prolactin (ng/ml) response to chlorpromazine stimulation of normal women without and with cyproheptadine pretreatment

| Case No. | No pretreatment                       |                                              |              | Cyproheptadine pretreatment           |                                              |             |
|----------|---------------------------------------|----------------------------------------------|--------------|---------------------------------------|----------------------------------------------|-------------|
|          | Pre-chlorpromazine level of prolactin | Highest prolactin value after chlorpromazine | Difference   | Pre-chlorpromazine level of prolactin | Highest prolactin value after chlorpromazine | Difference  |
| N1       | 22                                    | 77                                           | + 55 (+250%) | 31                                    | 27                                           | - 4 (- 12%) |
| N2       | 20                                    | 78                                           | + 58 (+290%) | 16                                    | 43                                           | +27 (+169%) |
| N3       | 13                                    | 24                                           | + 11 (+ 84%) | 15                                    | 23                                           | + 8 (+ 53%) |
| N4       | 21                                    | 33                                           | + 12 (+ 67%) | 17                                    | 21                                           | + 4 (+ 23%) |
| N5       | 26                                    | 129                                          | +103 (+396%) | 9                                     | 27                                           | +18 (+200%) |

cyproheptadine, 4 mg, was given every 6 hours for at least 2 days prior to the injection of chlorpromazine.

Serum prolactin was determined by the laboratory of the Clinical Research Center, by means of a double antibody radioimmunoassay.<sup>9</sup> The normal value for this laboratory is <30 ng/ml.

**Results**

In normal women, pretreatment with cyproheptadine inhibited the chlorpromazine-induced rise in serum prolactin, as shown in Table II. These results were statistically significant by the paired ttest (p = 0.07).

When similar studies were done in women with hyperprolactinemia and galactorrhea (Table III), there were no statistically significant differences in response to chlorpromazine either with or without cyproheptadine pretreatment.

**Comment**

In the normal women, chlorpromazine consistently produced a rise in prolactin, which resulted in an increase of at least 67%. If 50 instead of 25 mg of chlorpromazine had been used, the expected doubling of the levels of prolactin may have occurred in each instance.

When pretreatment with cyproheptadine was administered, the rise in prolactin was inhibited in each instance. This demonstrates that the serotonergic system has an excitatory effect on the release of prolactin, and that the administration of chlorpromazine did not result in as great an increase in prolactin as that observed when the serotonin system was intact.

The results in normal subjects could be explained by serum prolactin levels being proportional to the net effect of stimulation by TRF and inhibition by PIF. It



**Table III.** Serum prolactin (ng/ml) response to chlorpromazine stimulation of women with hyperprolactinemia and galactorrhea without and with cyproheptadine pretreatment

| Case No. | No pretreatment                       |                                              |              | Cyproheptadine pretreatment           |                                              |             |
|----------|---------------------------------------|----------------------------------------------|--------------|---------------------------------------|----------------------------------------------|-------------|
|          | Pre-chlorpromazine level of prolactin | Highest prolactin value after chlorpromazine | Difference   | Pre-chlorpromazine level of prolactin | Highest prolactin value after chlorpromazine | Difference  |
| 1.       | 91                                    | 194                                          | +103 (+113%) | 156                                   | 148                                          | - 8 (- 5%)  |
| 2.       | 83                                    | 200                                          | +117 (+140%) | 168                                   | 256                                          | + 88 (+52%) |
| 3.       | 47                                    | 70                                           | + 23 (+ 50%) | 69                                    | 102                                          | + 33 (+47%) |
| 4.       | 257                                   | 209                                          | - 33 (- 13%) | 161                                   | 209                                          | + 48 (+30%) |
| 5.       | 116                                   | 115                                          | - 1 (- 1%)   | 131                                   | 150                                          | + 19 (+15%) |
| 6.       | 163                                   | 213                                          | + 50 (+ 31%) | 144                                   | 160                                          | + 16 (+11%) |
| 7.       | 116                                   | 123                                          | + 7 (+ 6%)   | 142                                   | 132                                          | - 10 (- 7%) |
| 8.       | 148                                   | 186                                          | + 38 (+ 26%) | 146                                   | 190                                          | + 44 (+30%) |
| 9.       | 141                                   | 165                                          | + 24 (+ 17%) | 108                                   | 210                                          | +102 (+44%) |

can be speculated that, when chlorpromazine alone was administered, the TRF effect remained constant and the chlorpromazine induced a decrease in PIF that resulted in an increased serum prolactin. However, when TRF was also inhibited, there was less stimulating effect to be exerted against reduced PIF, so that serum prolactin rose less or even decreased. On the other hand, the serotonergic system may have directly affected the discharge of PIF. These mechanisms were suggested by rat studies done by Kamberi and associates.<sup>10</sup>

The hyperprolactinemic women were not subdivided on the basis of polytomographic examination into those with and those without findings compatible with microadenoma, because of recent reports that microadenomas may exist without polytomographic changes.<sup>11</sup> It is possible that all of the women with hyperprolactinemia had microadenomas, and also that some of the women with minimal sellar changes did not have a microadenoma.

In women with hyperprolactinemia, chlorpromazine, 25 mg, produced an additional rise of at least 67% in only seven of nine instances. Similar results showing a lack of increase when chlorpromazine or TRF was administered to persons with pituitary adenomas have been reported, thus suggesting that the pituitary is already secreting at maximum capacity, and neither increased TRF or decreased PIF can further stimulate the production of prolactin.<sup>12, 13</sup>

When cyproheptadine pretreatment was administered before chlorpromazine to hyperprolactinemic

women, no consistent difference in the response to chlorpromazine was noticed. Cases 1 and 2, in which normal elevations of prolactin followed chlorpromazine, also had this response to chlorpromazine inhibited by cyproheptadine in a manner similar to that seen in the normal controls. It is possible that in these two cases the pituitary was not secreting at maximum capacity and that, therefore, it was still regulated to some extent by PIF and TRF.

Our observations on the response of serum prolactin to the administration of chlorpromazine suggest that in normal women the serotonergic system is involved as an excitatory agent for the secretion of prolactin. In women with hyperprolactinemia and galactorrhea in whom the pituitary was already secreting at maximum capacity, there was little, if any, response to chlorpromazine, and pretreatment with a serotonin antagonist induced no significant changes in this response, so that the effects of the system could not be demonstrated in this situation.

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# Progesterone-induced smooth muscle-like cells in the subperitoneal nodules produced by estrogen

## Experimental approach to leiomyomatosis peritonealis disseminata

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In order to examine the pathogenesis and histogenesis of leiomyomatosis peritonealis disseminata (LPD), guinea pigs were treated with various doses of estradiol benzoate (E) (40, 80, 100, and 200  $\mu$ g/day, three times a week, by intramuscular injection). After treatment for 3 months, each dose of E produced lesions simulating the disseminated pattern of LPD. Ultrastructurally, the nodules were considered to be composed of cells resembling those of fibroblast. In guinea pigs, pretreatment with estradiol benzoate (100  $\mu$ g/day, three times a week intramuscularly for 3 months followed by combination treatment with E and progesterone (P) (0.5 and 1.0 mg/day, once a week, intramuscularly) for 1 to 3 months produced nodules similar to those with estrogen alone. Ultrastructurally, these nodules were composed of cells resembling smooth muscle and decidual cells. According to these results, we conjectured that estrogen differentiates and proliferates cells with the features of fibroblast-like cells from the subcoelomic totipotent mesenchyme, and these cells are differentiated into smooth muscle-like cells and decidual-like cells under the influence of both estrogen and progesterone, resulting in the production of multiple subperitoneal nodules. (AM. J. OBSTET. GYNECOL. 139:164, 1981.)

IN RECENT YEARS an abnormality has been described that is characterized by the development of multiple nodules of variable sizes, scattered all over the peritoneal cavity, and representing the histologic characteristics of leiomyoma. It has, therefore, been called "leiomyomatosis peritonealis disseminata"<sup>1-6</sup> (LPD). LPD is an entity whose histologic and clinical peculiarities re-

main unclear. Association with pregnancy<sup>2, 3, 5, 6</sup> and, at a later age, with an ovarian tumor<sup>1</sup> prone to endocrine activity, however, strongly suggest that there is a link between sex hormones and the development of LPD.<sup>1-6</sup>

Parmley, Winn, and co-workers<sup>2, 6</sup> recently hypothesized that LPD lesions are fibroid tumors resulting from a decidual reaction throughout the peritoneal cavity. However, while fibrosis of a decidual reaction may have occurred, these cases of LPD demonstrated characteristic features of smooth muscle.<sup>3, 5</sup> Our previous report of LPD<sup>5</sup> demonstrated that larger tumors in the omentum comprised smooth muscle cells, but smaller tumors in the omentum consisted of fibroblasts and cells resembling decidual cells and there was a relative hyperestrogenism during pregnancy as deter-

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mined by analysis of sex steroids. These observations agree somewhat with the theory of Parmley, Winn, and co-workers,<sup>2, 6</sup> but their theory cannot explain smooth muscle-cell proliferation. In our case, we conjectured that relative hyperestrogenism initiated the growth of fibroids as a result of a decidual reaction throughout the peritoneal cavity; this was probably followed by the development of smooth muscle from the fibroblasts of these fibroids through progesterone action.<sup>5</sup>

Animal experiments<sup>8</sup> performed in an attempt to understand the histogenesis of uterine leiomyomas have resulted in the production of lesions simulating the disseminated pattern of LPD. Multiple peritoneal fibroids were produced when guinea pigs were treated with large doses of estrogens, and the nodules regressed when the steroid stimulant was discontinued.<sup>9</sup> Both fibroblasts and a few smooth muscle cells were described as the constitutional elements of these experimental nodules.<sup>8</sup> Unfortunately, however, in an editorial in *Lancet*,<sup>10</sup> this experiment was not considered to represent the formation of smooth muscle nodules.

The present study was designed to produce LPD-like lesions in guinea pigs by prolonged administration of various doses of estrogen with electron microscopic identification of the nodules. The guinea pigs were then given the combination treatment with estrogen and progesterone and the nodules were again studied by electron microscopy. Through these studies, we tried to verify the possibility that estrogen initiates the growth of fibroids, followed by the differentiation of the fibroblast-like cells into smooth muscle through the action of progesterone.

#### Material and methods

A total number of 320 young female guinea pigs (Hartree line) weighing 300 to 400 gm were used in the experiments.

We used 150 guinea pigs for preliminary studies. Oophorectomy was performed by simple lumbar sections in half of them following administration of intramuscular ketamine. Both oophorectomized and nonoophorectomized animals were treated according to the following preliminary experimental schedules: group 1, control animals; group 2, control animals treated with the solvent of estradiol benzoate (E) (0.2, 0.4, 0.5, and 1.0 ml/day) three times a week for 2 to 6 months by intramuscular injection; group 3, animals treated with various doses of E (40, 80, 100, and 200  $\mu$ g/day), three times a week for 2 to 6 months, by intramuscular injection.

These preliminary studies revealed that nodules were produced in the abdominal cavity in each animal

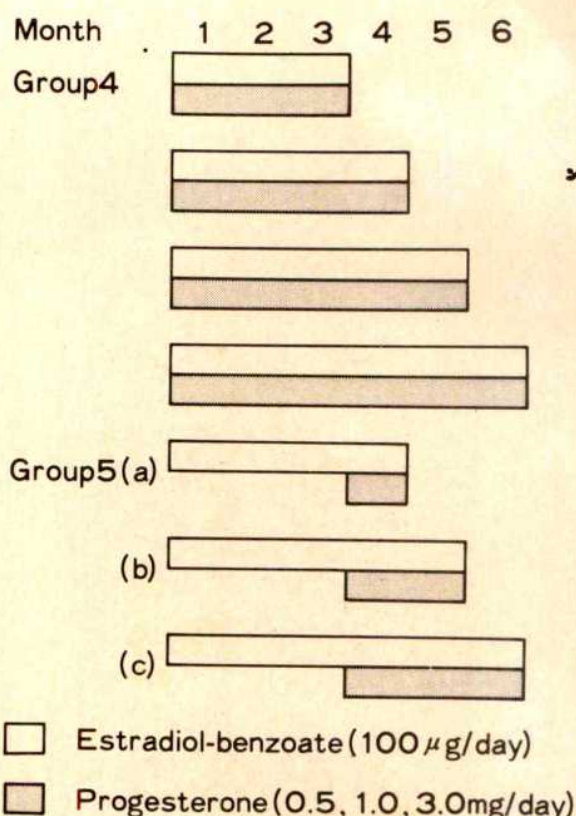


Fig. 1. Schedules of the combination treatments with estrogen and progesterone.

(group 3) during a minimum of 3 months' treatment with various doses of E, regardless of oophorectomy; there were no differences in the production rate and the characteristics of the nodules.

We used 170 animals for the following combination treatment with estrogen and progesterone. From the preliminary studies, it was decided to inject E, 100  $\mu$ g/day, three times a week, in nonoophorectomized animals, and three doses of progesterone (P) (0.5, 1.0, and 3.0 mg/day) were injected intramuscularly once a week (Fig. 1).

In group 4, we used combined treatment with E, 100  $\mu$ g/day, three times a week, intramuscularly, and various doses of P, 0.5, 1.0, and 3.0 mg/day, once a week, intramuscularly, for 3 to 6 months.

In group 5a, we administered pretreatment with E for 3 months, followed by the combination treatment with E and three doses of P for 1 month. In group 5b, we administered pretreatment with E for 3 months, followed by the combination treatment with E and three doses of P for 2 months. In group 5c, we administered pretreatment with E for 3 months, followed by the combination treatment with E and three doses of P for 3 months.



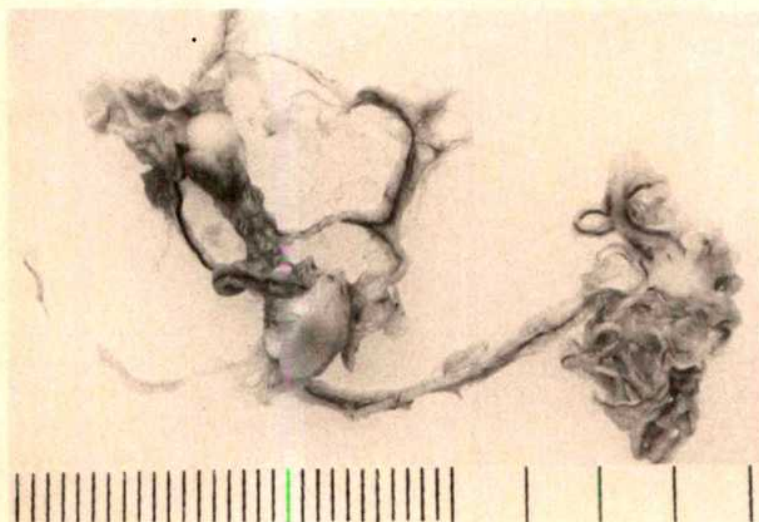


**Fig. 2.** Multiple nodules within omentum, produced in nonoophorectomized guinea pig, after treatment with E (100 µg/day) for 4 months. Gross appearance of nodules is considered to simulate clinical LPD.

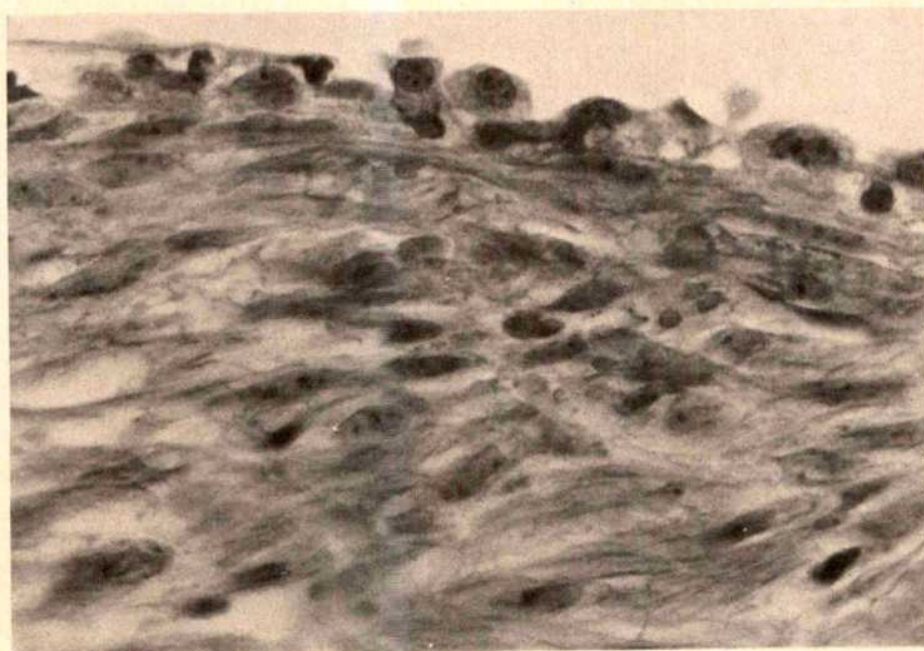


**Fig. 3.** Section of omental nodule produced by estrogen treatment alone demonstrates proliferation of spindle-shaped cells under the coelomic epithelium. (Gomori's trichrome.  $\times 400$ .)

In group 6, three doses of progesterone treatment were administered for 3 to 6 months.

Immediately after the animals were killed by ether inhalation, the abdominal cavity was carefully observed, then the uterus, omentum, and nodules were dissected out. Tissues mainly from the nodules in the omentum were promptly fixed in chilled 6.25% glutaraldehyde buffered with 0.1M cacodylate buffer (pH

7.4) and then in 1.0% osmium tetroxide solution. They were stained en bloc in uranyl acetate solution, dehydrated in a series of graded ethanols followed by propylene oxide, and embedded in Epon 812. Ultrathin sections were obtained with a glass knife on a Porter-Blum MT2B ultramicrotome, stained by lead citrate solution, and examined with a Hitachi HU-11D at 75 kV. The remaining materials were fixed in 10% neutral



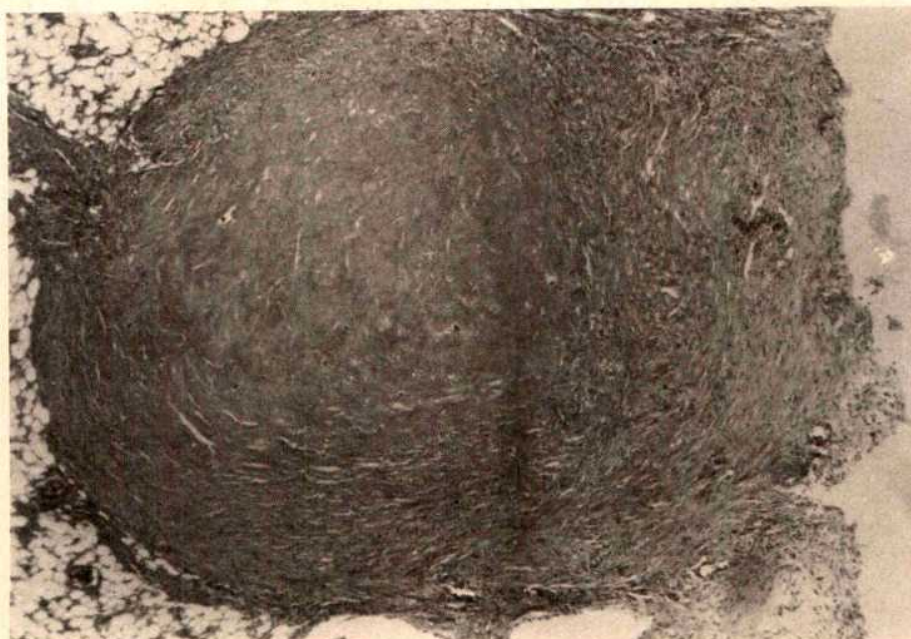


**Fig. 4.** Section of omental nodule produced by estrogen treatment alone demonstrates spindle-shaped cells and collagen-like materials constructing a whorled appearance of the nodule. (Gomori's trichrome.  $\times 40$ .)

formalin for light microscopic studies and stained by hematoxylin-eosin and Gomori's trichrome, respectively.

### Results

**Results produced by estrogen treatment.** The animals (group 3) had multiple nodules in the abdominal cavities, particularly on the surfaces of the uterine horn, peritoneum, spleen, and pancreas and in the omentum (Fig. 2) and mesocolon. Every dose given of E (40, 80, 100, and 200  $\mu\text{g}/\text{day}$ ) produced these lesions. After treatment for 3 months, the nodules were observed in all animals regardless of whether oophorectomy had been performed, but no nodules were observed in the control animals (groups 1 and 2).

The gross appearance of the nodules was characterized by a hemispherical shape on the uterus and peritoneum and a round shape in the omentum (Fig. 2) and mesocolon. Cut surfaces of these nodules were gray-white, with a slightly whorled appearance and with an elastic, hard consistency.

Microscopically, every nodule showed the proliferative features of spindle-shaped cells under the coelomic epithelium (Fig. 3). These spindle-shaped cells and collagen-like materials constructed a whorled appearance of the nodules (Fig. 4). In some spindle-shaped cells the cytoplasm was stained slightly red, although almost all were stained scarcely red by Gomori's tri-

chrome stain (Fig. 3). At electron microscopic examination, these omental nodules contained cells with increased chromatin in the nucleus and had a scanty filamentous structure; abundant rough endoplasmic reticulum and mitochondria occupied the cytoplasm (Fig. 5). Few pinocytotic vesicles were observed along the cytoplasmic membranes. These observations indicate that the nodules produced by estrogen treatment were composed of cells resembling fibroblast.

**Results produced by both estrogen and progesterone treatment.** In the animals pretreated with estrogen for 3 months, followed by a combination treatment with estrogen and progesterone (group 5), at doses of 0.5 and 1.0 mg of progesterone per day, multiple nodules were produced in the abdominal cavities similar to those with estrogen treatment alone. No nodules were observed in groups 4 and 6 or in group 5, in which a dose of 3.0 mg of progesterone per day was used. The production rate of the nodules was approximately 75% in the animals treated at doses of 0.5 and 1.0 mg of progesterone per day (group 5).

The nodules grossly appeared smaller and showed less tendency to adhere to the neighboring organs than those produced by estrogen alone.

Microscopically, the nodules in group 5 animals showed almost the same features as these produced by estrogen alone. However, the spindle-shaped cells were slightly larger and longer than those of estrogen-



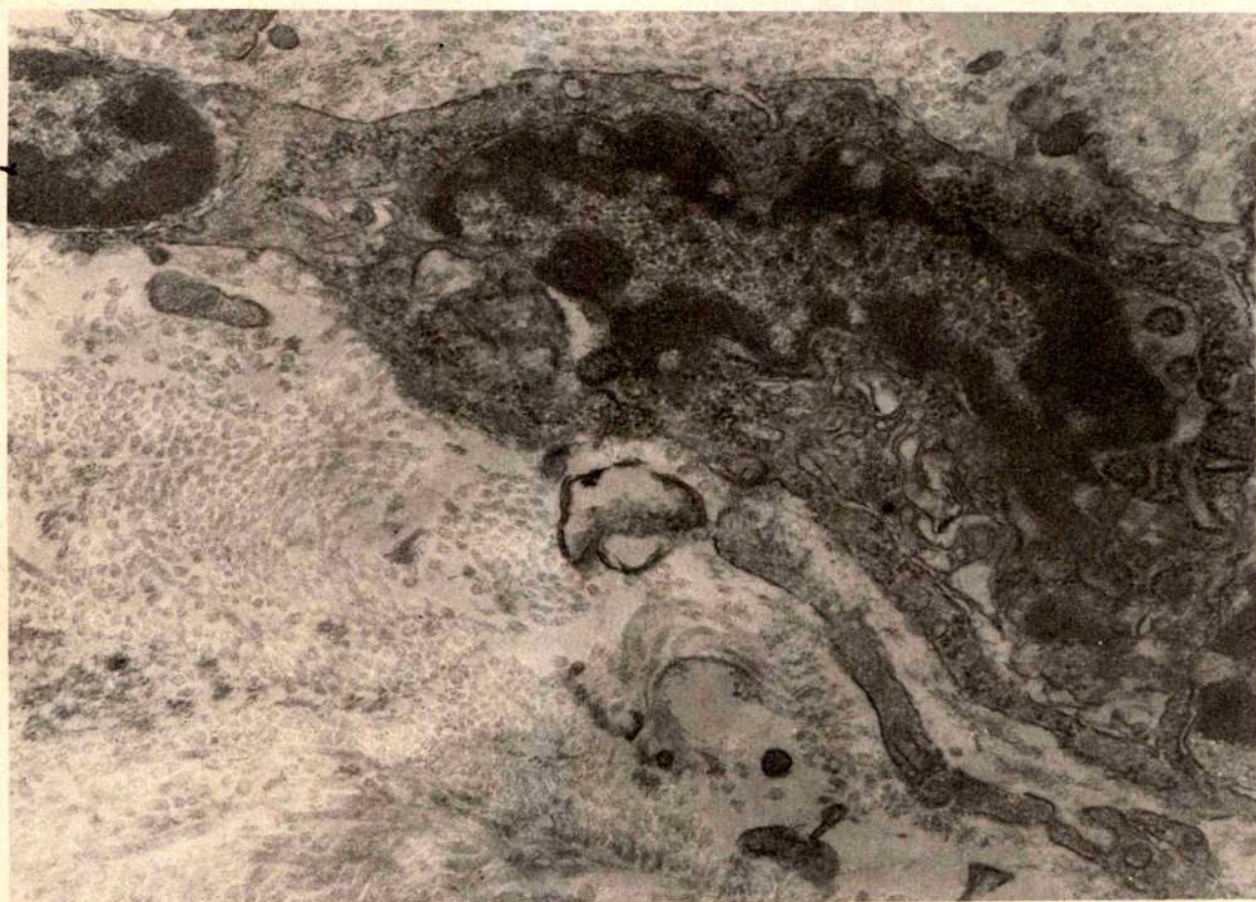


**Fig. 5.** Electron micrograph of omental nodule, produced by estrogen treatment alone, demonstrating increased chromatin in the nucleus and scanty filamentous structures as well as abundant rough endoplasmic reticulum and mitochondria. (Original magnification  $\times 26,000$ .)

treated nodules and were stained red by Gomori's trichrome method (Fig. 6). Some large, spindle-shaped cells were also observed in the same nodules, and their cytoplasm stained slightly red by Gomori's trichrome method.

The omental sections of group 4 animals (treated with 0.5 and 1.0 mg of progesterone per day) revealed a solitary mass of spindle-shaped cells stained red by Gomori's trichrome beneath the coelomic epithelium, and these cells were considered to be the cells resembling smooth muscle cells (Fig. 7). Serial sections confirmed that they were not derived from vascular smooth muscle cells.

By electron microscopy, myofilamentous structures in the cytoplasm as well as rough endoplasmic reticulum and mitochondria located in the perinuclear region were demonstrated in the omental nodules (group 5). Well-developed pinocytotic vesicles were seen along the cytoplasmic membranes (Fig. 8). These

features closely resembled the ultrastructural characteristics of smooth muscle cells. Moreover, examination also revealed cells that had abundant microfilaments with rough endoplasmic reticulum and vacuoles in the cytoplasm (Fig. 9). Pinocytotic vesicles were seldom seen along the cytoplasmic membranes and some parts of the membrane showed ill-defined areas between the intracytoplasmic microfilaments and the extracellular collagen fibers. These cells were considered to be the cells having some characteristic features of decidual cells.<sup>11</sup> From these observations, it appears that the nodules consisted of both smooth muscle-like cells and decidual-like cells.

Results of both treatment regimens indicate that fibroblast-like cells were observed in the nodules treated with estrogen alone and smooth muscle-like cells and decidual-like cells were observed in the nodules treated with both estrogen and low doses of progesterone.



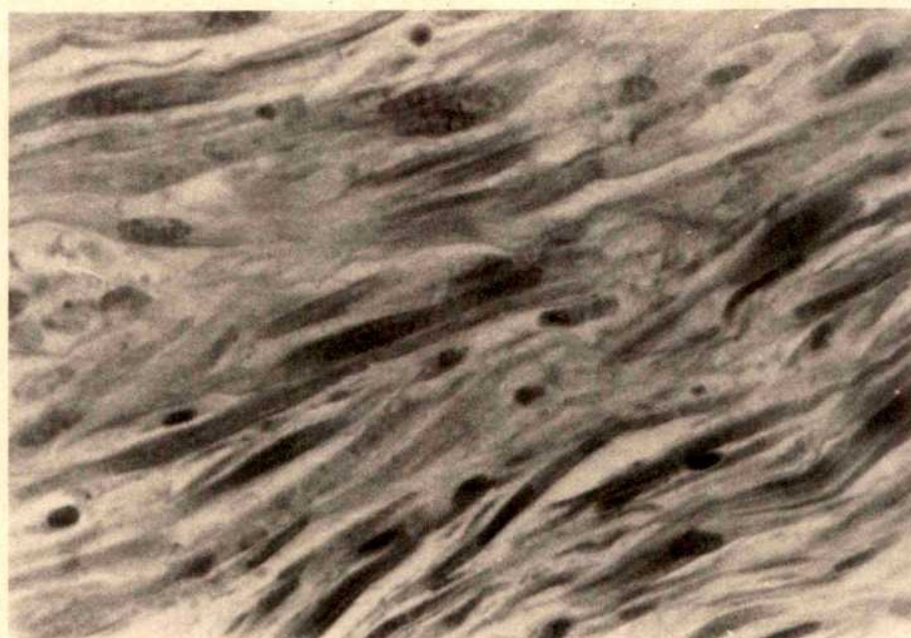


**Fig. 6.** Section of omental nodule produced by estrogen pretreatment followed by combination treatment with estrogen and progesterone, demonstrating the smooth muscle-like cell proliferation with varying cellularity. (Gomori's trichrome.  $\times 400$ .)

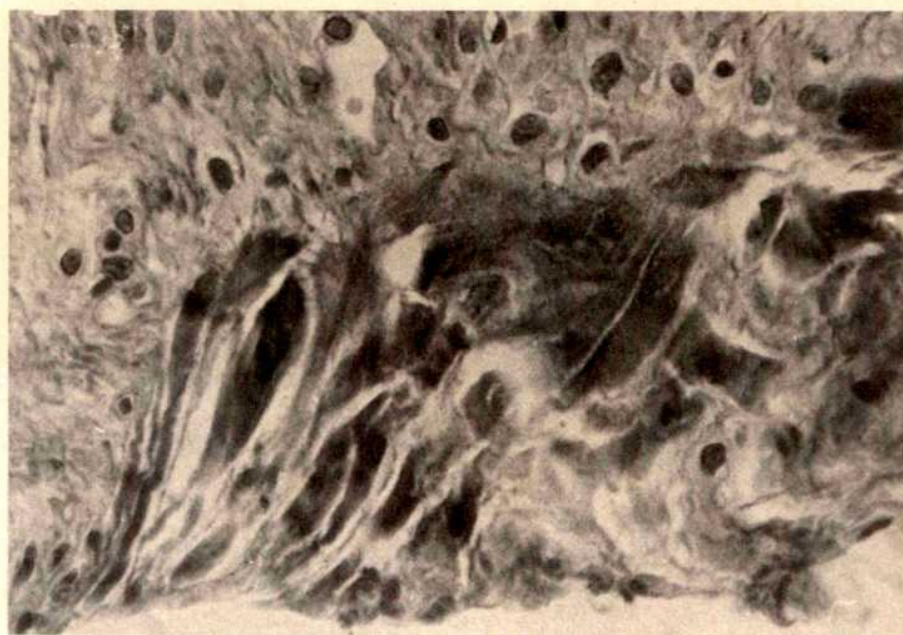


**Fig. 7.** Section of omentum treated with estrogen and progesterone, demonstrating a mass of smooth muscle-like cells in the proliferation of subcoelomic mesenchyme. (Gomori's trichrome.  $\times 400$ .)





**Fig. 8.** Electron micrograph of omental nodule, produced by estrogen pretreatment followed by combination treatment with estrogen and progesterone, demonstrating myofilamentous structure in cytoplasm, rough endoplasmic reticulum, and mitochondria located in perinuclear region. Well-developed pinocytotic vesicles can be seen. (Original magnification  $\times 26,000$ .)

### Comment

In the abdominal cavity of the guinea pig, our experiments produced the lesions simulating the disseminated pattern of LPD by prolonged administration of estrogen: Multiple nodules of various sizes were scattered on the surfaces of the uterine horn, peritoneum, spleen, and pancreas and in the omentum and mesocolon. The scattered pattern and gross appearance of our experimental nodules produced by estrogen were quite similar to those of Lipshutz and Vargas.<sup>8</sup>

The nodules produced by estrogen were composed of cells resembling fibroblast or immature smooth muscle cells, and all nodules were covered with intact coelomic epithelium. Therefore, the nodules were considered to be the results of the proliferation of subcoelomic mesenchyme. Ultrastructurally, the filamentous structures, considered to be one of the characteristic features of immature smooth muscle<sup>12</sup> or so-called

myofibroblast,<sup>13, 14</sup> could not be demonstrated in these cells and thus identified as cells resembling fibroblast.

The nodules produced by pretreatment with estrogen followed by the combination treatment of estrogen and low doses of progesterone were composed of cells resembling smooth muscle cells, cells with some features of decidual cells,<sup>11</sup> and collagen by electron microscopic studies. It was mentioned that the embryonic kinship between the müllerian duct structures and the peritoneum is reflected in the similarity of the responses of the peritoneum and its underlying stroma to the response of the endometrium when stimulated with estrogen and progesterone.<sup>2</sup> Therefore, our experimental nodules themselves are considered to be the results of subcoelomic mesenchymal proliferation through the actions of both estrogen and progesterone.

It has been suggested that fibroblasts can differentiate into smooth muscle cells in studies of vascular





**Fig. 9.** Electron micrograph of omental nodule, produced by estrogen pretreatment followed by combination treatment with estrogen and progesterone, demonstrating abundant microfilaments, rough endoplasmic reticulum, and vacuoles in cytoplasm. Pinocytotic vesicles are rare. ( $\times 30,000$ .)

smooth muscle,<sup>15</sup> granulation tissue,<sup>13</sup> and the theca externa of the ovarian follicle.<sup>14</sup> However, recent clinical reports of the omental nodules in LPD and adenomyoma of the ileum<sup>16</sup> have described the presence of endometrial glands and stroma as well as smooth muscle cells. Moreover, many reports of LPD<sup>3, 5, 7</sup> have demonstrated the coexistence of smooth muscle cells and decidua-like cells. These findings imply that subcoelomic mesenchyme has a totipotent capacity of differentiating into smooth muscle cells and endometrial stroma as well as glands.<sup>4, 16</sup> The coexistence of the smooth muscle-like cells and the decidua-like cells in our experimental nodules is similar to that of these clinical pictures. Therefore, we conjecture that estrogen differentiates and proliferates the cells of the features of fibroblast-like cells from subcoelomic totipo-

tential mesenchyme and that they are differentiated into smooth muscle-like cells and decidua-like cells under the influence of both estrogen and progesterone, resulting in the production of multiple subperitoneal nodules.

In conclusion, our experimental observations have convinced us that clinical LPD may be the transient lesions produced by changes in the sex steroid milieu, particularly because the presence of hyperestrogenism and progesterone may act as an important factor in the production of smooth muscle cells and decidua-like cells in the subperitoneal nodules. In the future, these experimental nodules composed of smooth muscle-like cells may provide information that will be helpful in further studies of the histogenesis and pathogenesis of uterine leiomyomas.

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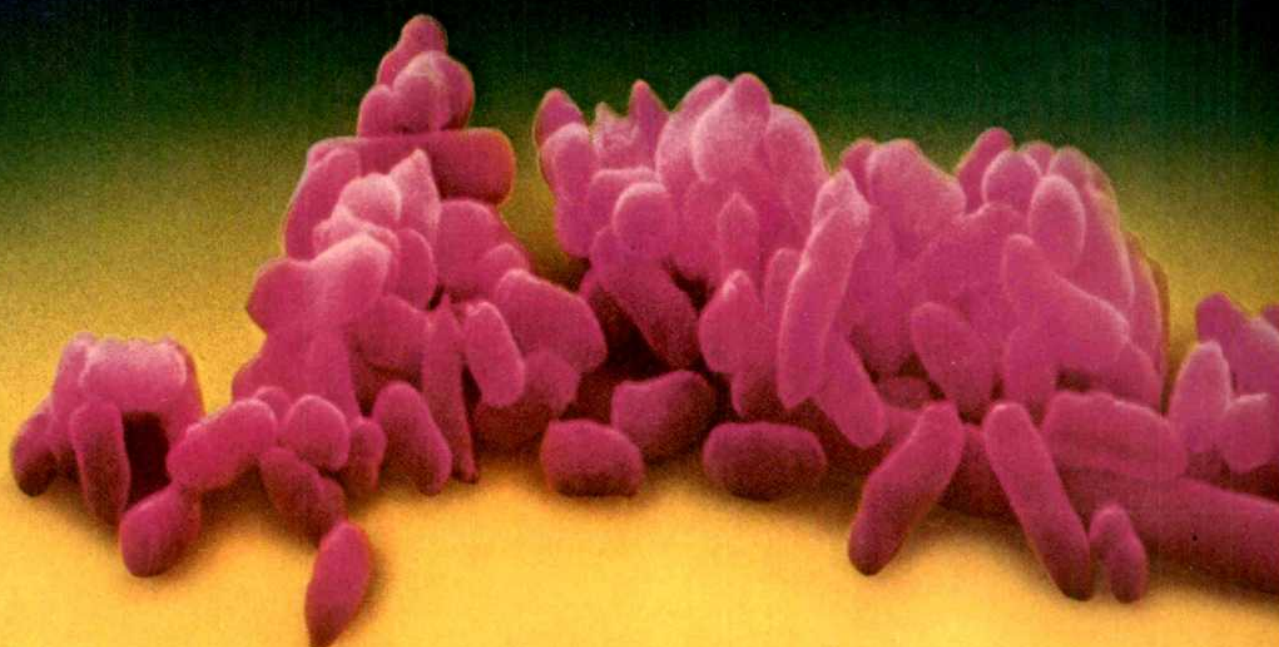
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From Searle-

# **“AN IMPORTANT THERAPEUTIC ADVANCE” against anaerobic infections**

\*Sydney M. Finegold, M.D., Professor of Medicine, UCLA School of Medicine; Chief, Infectious Disease Section, Wadsworth VA Medical Center, Los Angeles, California



Scanning electron micrograph of *Bacteroides fragilis* magnified 24,000 times and color enhanced.

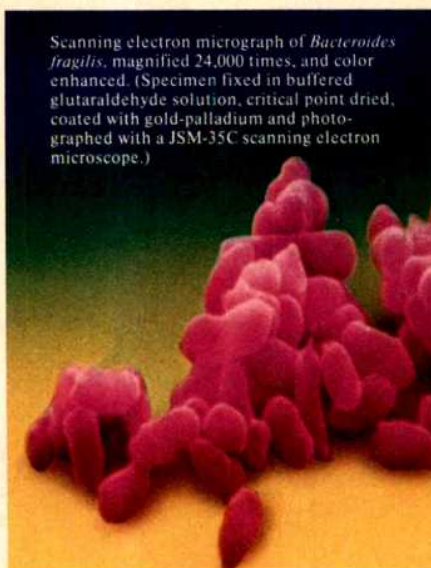


## Important News from Searle

### Announcing

# FLAGYL I.V.<sup>™</sup> (metronidazole HCl), a systemic anaerobicide highly effective against *Bacteroides fragilis*.

Flagyl I.V. is indicated (see indications and warnings) for treatment of serious infections due to *B. fragilis*, which is generally resistant to penicillin G, ampicillin, and most other antibiotics. In a mixed aerobic and anaerobic infection, antibiotics appropriate for the treatment of the aerobic infection should be used in addition to Flagyl I.V.



*B. fragilis* is often pathogenic in infections occurring below the diaphragm, and sometimes is pathogenic in infections above the diaphragm, such as brain and lung abscess, meningitis, aspiration pneumonia, and endocarditis. Flagyl I.V. is indicated in all of these, and has been found to be effective in *B. fragilis* infections not responding to clindamycin, chloramphenicol, or penicillin. See Table 1.

Table 1.\*

| CONDITIONS                                                                                                                     | SUSCEPTIBLE ANAEROBES                                              |                                                                                                                                                                            |                                   |                            |                              |                            |
|--------------------------------------------------------------------------------------------------------------------------------|--------------------------------------------------------------------|----------------------------------------------------------------------------------------------------------------------------------------------------------------------------|-----------------------------------|----------------------------|------------------------------|----------------------------|
|                                                                                                                                | <i>Bacteroides</i> species, including the <i>B. fragilis</i> group | <i>Clostridium</i> species                                                                                                                                                 | <i>Peptostreptococcus</i> species | <i>Peptococcus</i> species | <i>Fusobacterium</i> species | <i>Eubacterium</i> species |
| INTRA-ABDOMINAL INFECTIONS, including peritonitis, intra-abdominal abscess, liver abscess                                      | ✓                                                                  | ✓                                                                                                                                                                          | ✓                                 | ✓                          |                              | ✓                          |
| SKIN AND SKIN STRUCTURE INFECTIONS                                                                                             | ✓                                                                  | ✓                                                                                                                                                                          | ✓                                 | ✓                          | ✓                            |                            |
| GYNECOLOGIC INFECTIONS, including endometritis, endomyometritis, tubo-ovarian abscess, and postsurgical vaginal cuff infection | ✓                                                                  | ✓                                                                                                                                                                          | ✓                                 | ✓                          |                              |                            |
| BACTERIAL SEPTICEMIA                                                                                                           | ✓                                                                  | ✓                                                                                                                                                                          |                                   |                            |                              |                            |
| BONE AND JOINT INFECTIONS, as adjunctive therapy                                                                               | ✓                                                                  | *Based on Flagyl I.V. <sup>™</sup> (metronidazole HCl)<br>Clinical Studies: Susceptible Anaerobes and<br>Conditions Frequently Caused by One or<br>More of These Organisms |                                   |                            |                              |                            |
| CNS INFECTIONS, including brain abscess and meningitis                                                                         | ✓                                                                  |                                                                                                                                                                            |                                   |                            |                              |                            |
| LOWER RESPIRATORY TRACT INFECTIONS, including pneumonia, empyema, and lung abscess                                             | ✓                                                                  |                                                                                                                                                                            |                                   |                            |                              |                            |
| ENDOCARDITIS                                                                                                                   | ✓                                                                  |                                                                                                                                                                            |                                   |                            |                              |                            |



## Important News from Searle

# FLAGYL I.V.<sup>™</sup> (metronidazole HCl) is effective against a wide range of anaerobes that often cause serious infections.

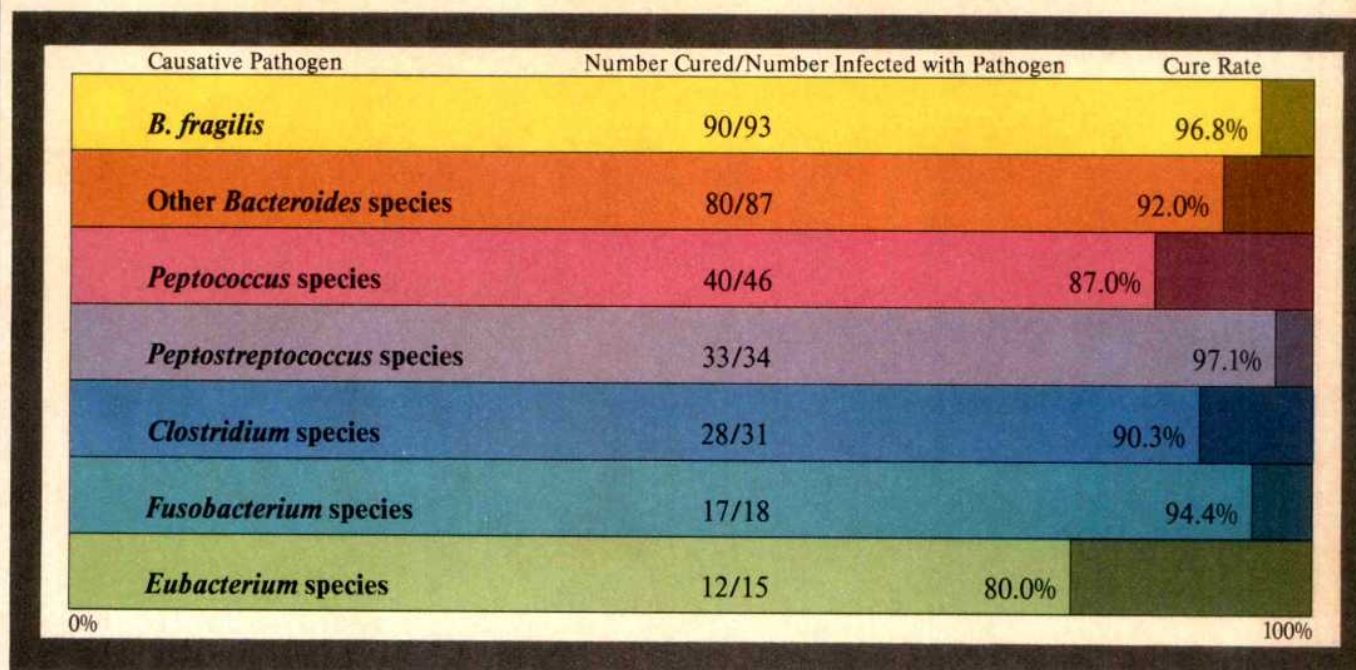
■ Metronidazole is "... consistently bactericidal against anaerobes."<sup>1</sup>

■ Metronidazole readily crosses the blood-brain barrier.

■ Metronidazole achieves excellent tissue penetration.

The chart below\* shows the effectiveness of Flagyl I.V. against major anaerobes in clinical studies involving 180 patients. (The anaerobes and conditions are listed in Table 1.)

Patients Cured with Flagyl I.V.<sup>™</sup> (metronidazole HCl) by Type of Pathogen



NOTE: The above chart is based on pooled data from two clinical studies representing a total of 180 patients with various kinds of infections, including: intra-abdominal, skin and skin structure, gynecologic, bone and joint, CNS,

and pleuropulmonary infections; bacterial septicemia; and endocarditis. Of the 180 patients, 106 (58.9%) were infected with more than one anaerobe.

## SEARLE

\*Data on file, G. D. Searle & Co.

Please see brief summary of prescribing information on last page of this advertisement.



## Important News from Searle

**In clinical trials with  
FLAGYL I.V.<sup>TM</sup> (metronidazole HCl),  
the frequency and type of adverse  
reactions followed the pattern of  
17 years' previous experience  
with oral metronidazole.**

**FLAGYL I.V.  
is effective and relatively safe.**

- No cases of pseudomembranous colitis have been reported to date with Flagyl I.V.
- No cases of aplastic anemia have been reported.
- Although phlebitis has been reported, this reaction can be avoided or minimized by proper I.V.-admixture and -administration procedures.
- Metronidazole has been shown to be carcinogenic in mice and rats. The relevance of this finding with regard to humans has not been demonstrated. See prescribing information for *Warnings* and *Indications*.

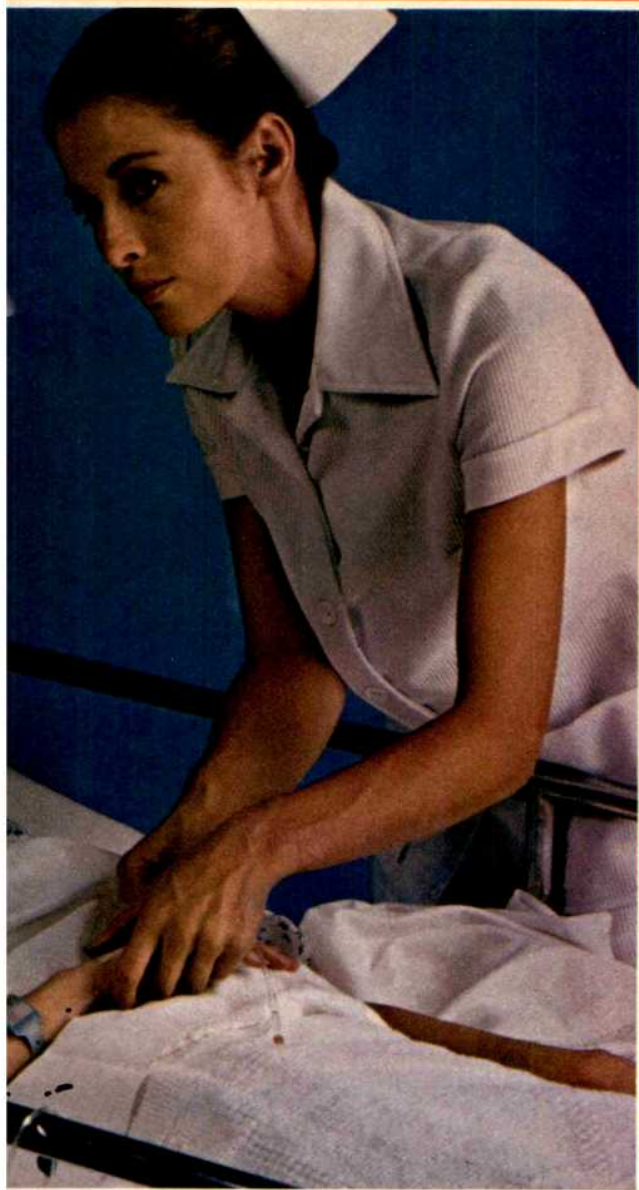
For information concerning adverse reactions, consult the brief summary of prescribing information on the last page of this advertisement.





## Important News from Searle

**Drug-induced resistance to  
FLAGYL I.V.<sup>TM</sup> (metronidazole HCl)  
has not been documented,  
and the development of  
significant resistance  
appears unlikely to occur.**



■ Although specific anaerobes may be naturally resistant to Flagyl I.V., drug-induced resistance has not been documented.

■ No drug-induced resistance was reported in Flagyl I.V. clinical trials.

■ Oral metronidazole usage (for trichomoniasis and amebiasis), in over 20 million patients and for more than 17 years in the United States alone, has not resulted in the reported development of drug resistance in anaerobic bacteria.

■ Drug-induced resistance appears unlikely to occur because of the mechanism of action of Flagyl I.V.

**Reference:**

1. Finegold, S.M., Antimicrobial Therapy of Anaerobic Infections: A Status Report, Hosp. Pract. 14:71-81 (Oct.) 1979.

**SEARLE**

Please see brief summary of prescribing information on next page.



# FLAGYL I.V.<sup>TM</sup> (metronidazole HCl)

## Flagyl I.V.<sup>TM</sup> (metronidazole HCl)

### WARNING

Metronidazole has been shown to be carcinogenic in mice and rats (see Warnings). Its use, therefore, should be reserved for serious anaerobic infections where, in the judgment of the physician, the benefit outweighs the possible risk.

**Indications and Usage:** Flagyl I.V. is indicated in the treatment of serious infections caused by susceptible anaerobic bacteria. Indicated surgical procedures should be performed in conjunction with Flagyl I.V. therapy. In a mixed aerobic and anaerobic infection, antibiotics appropriate for the treatment of the aerobic infection should be used in addition to Flagyl I.V.

Flagyl I.V. has been found to be effective in *Bacteroides fragilis* infections resistant to clindamycin, chloramphenicol, and penicillin.

**INTRA-ABDOMINAL INFECTIONS**, including peritonitis, intra-abdominal abscess and liver abscess, caused by *Bacteroides* species including the *B. fragilis* group (*B. fragilis*, *B. distasonis*, *B. ovatus*, *B. theta*, *B. distaomicon*, *B. vulgatus*), *Clostridium* species, *Eubacterium* species, *Peptococcus* species, and *Peptostreptococcus* species.

**SKIN AND SKIN STRUCTURE INFECTIONS** caused by *Bacteroides* species including the *B. fragilis* group, *Clostridium* species, *Peptococcus* species, *Peptostreptococcus* species, and *Fusobacterium* species.

**GYNECOLOGIC INFECTIONS**, including endometritis, endomyometritis, tubo-ovarian abscess, and post-surgical vaginal cuff infection, caused by *Bacteroides* species including the *B. fragilis* group, *Clostridium* species, *Peptococcus* species, and *Peptostreptococcus* species.

**BACTERIAL SEPTICEMIA** caused by *Bacteroides* species including the *B. fragilis* group, and *Clostridium* species.

The following conditions when caused by *Bacteroides* species including the *B. fragilis* group:

**BONE AND JOINT INFECTIONS**, as adjunctive therapy

**CNS INFECTIONS**, including meningitis and brain abscess

**LOWER RESPIRATORY TRACT INFECTIONS**, including pneumonia, empyema, and lung abscess

**ENDOCARDITIS**

**Contraindications:** Prior history of hypersensitivity to metronidazole or other nitroimidazole derivatives.

**Warnings:** Convulsive seizures and peripheral neuropathy have been reported in patients treated with metronidazole. The appearance of abnormal neurologic signs demands the prompt evaluation of the continuation of the drug.

Metronidazole has shown evidence of carcinogenicity in studies involving chronic, oral administration in mice and rats, but similar studies in hamsters were negative. It has also shown mutagenicity in some *in vitro* assays, but *in vivo* studies did not show a potential for genetic damage.

**Precautions:** Since patients with severe hepatic disease metabolize metronidazole slowly with resultant accumulation of metronidazole and its metabolites in the plasma, doses below those usually recommended should be administered cautiously. Known or previously unrecognized candidiasis may present more prominent symptoms during therapy and requires treatment with a candidal agent.

Use with care in patients with evidence of or history of blood dyscrasia. Mild leukopenia has been observed during metronidazole administration; however, no persistent hematologic abnormalities attributable to metronidazole have been observed in clinical studies. Total and differential leukocyte counts are recommended before and after therapy.

Metronidazole has been reported to potentiate the anticoagulant effect of warfarin and other oral coumarin anticoagulants resulting in a prolongation of prothrombin time and this should be considered when Flagyl I.V. is prescribed for patients on such anticoagulant therapy.

Avoid alcoholic beverages during metronidazole therapy.

Metronidazole may interfere with certain chemical analyses for serum glutamic oxalacetic transaminase resulting in decreased values.

Safe use in pregnancy has not been established. Metronidazole crosses the placental barrier and enters the fetal circulation rapidly. This drug should be used during pregnancy only if clearly needed.

Metronidazole is secreted in breast milk in concentrations similar to those found in plasma. If use of Flagyl I.V. is deemed essential, an alternative method of infant feeding should be used.

Safety and effectiveness in children have not been established.

**Adverse Reactions:** Convulsive seizures, nausea, vomiting, abdominal discomfort, diarrhea, unpleasant metallic taste, reversible neutropenia (leukopenia), erythematous rash, pruritus, headache, dizziness, syncope, fever, darkened urine, and thrombophlebitis after intravenous infusion that can be minimized or avoided by not prolonging use of indwelling intravenous catheters. Since persistent peripheral neuropathy has been reported in some patients receiving prolonged metronidazole therapy, patients should be observed carefully if neurologic symptoms occur and a prompt evaluation made of the benefit/risk ratio of the continuation of therapy.

Additional adverse reactions have been reported during treatment with oral metronidazole: anorexia, epigastric distress, constipation; furry tongue, glossitis, and stomatitis sometimes with a sudden overgrowth of *Candida*; flattening of the T-wave in electrocardiographic tracings; vertigo, incoordination, ataxia, confusion, irritability, depression, weakness, insomnia, urticaria, flushing, nasal congestion, dryness of mouth (or vagina or vulva), dysuria, cystitis, polyuria, incontinence, a sense of pelvic pressure, proliferation of *Candida* in the vagina, dyspareunia, decrease of libido, proctitis, and fleeting joint pains.

### Dosage and Administration:

**Adults:**

**Loading Dose**—15 mg/kg infused over one hour (approximately 1 g for a 70-kg adult).

**Maintenance Dose**—7.5 mg/kg infused over one hour every six hours (approximately 500 mg for a 70-kg adult). The first maintenance dose should be instituted six hours following the initial loading dose.

Parenteral therapy may be changed to oral Flagyl<sup>®</sup> (metronidazole) when conditions warrant, based upon the severity of the disease and the response of the patient to Flagyl I.V. treatment. The usual adult oral dosage is 7.5 mg/kg every six hours. A maximum of 4.0 g should not be exceeded during a 24-hour period.

Patients with severe hepatic disease metabolize metronidazole slowly, with resultant accumulation of metronidazole and its metabolites in the plasma. Accordingly, for such patients, doses below those usually recommended should be administered cautiously. Close monitoring of plasma metronidazole levels and toxicity is recommended.

The dose of Flagyl I.V. should not be specifically reduced in anuric patients since accumulated metabolites may be rapidly removed by dialysis.

The usual duration of therapy is 7 to 10 days; however, infections of the bone and joint, lower respiratory tract, and endocardium may require longer treatment.

Flagyl I.V. is to be administered by slow intravenous drip infusion only, either as a continuous or intermittent infusion. I.V. admixtures containing Flagyl I.V. and other drugs should be avoided. If used with a primary intravenous fluid system, the primary solution should be discontinued during infusion. Flagyl I.V. cannot be given by direct intravenous injection (I.V. bolus) because of the low pH (0.5 to 2.0) of the reconstituted product. FLAGYL I.V. MUST BE FURTHER DILUTED AND NEUTRALIZED FOR I.V. INFUSION.

**How Supplied:** Flagyl I.V. (metronidazole HCl) is supplied in single-dose lyophilized vials each containing 500 mg metronidazole equivalent, and 415 mg mannitol.

Searle Pharmaceuticals Inc.  
Box 5110, Chicago, Illinois 60680

OF1

# SEARLE



## Gestational age assessment

### I. Analysis of individual clinical observations

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MEL L. BARCLAY, M.D.

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Accurate assessment of the estimated date of confinement is a cornerstone of obstetric care. Traditionally this date has been predicted from historical and clinical examinations; however, there have been few studies of the accuracy of such predictions. Multiple estimators of gestational age were examined in 418 patients who were delivered of infants weighing  $\geq 3,000$  gm, following spontaneous onset of labor. Mean intervals from an event to delivery were calculated for last menstrual period (284.2 days), quickening (156.3 days), first audible fetal heart tones (136.2 days), uterine fundus at the umbilicus (140.8 days), and measurements of the fundal height. The variability in each of these estimators was examined and compared. The last menstrual period, if known with certainty, is the most accurate estimator, followed by the uterus at the umbilicus, first heard fetal heart tones, fundal height, and quickening. (AM. J. OBSTET. GYNECOL. 139:173, 1981.)

ONE OF THE MAJOR OBJECTIVES of antenatal obstetric care is the determination of the estimated date of confinement (EDC) and thereby the current estimated gestational age (EGA). Accurate knowledge of gestational age is indispensable to decision-making regarding diagnostic and/or therapeutic interventions during pregnancy. As our ability to intervene in the course of pregnancy has expanded, the necessity for

accurate dating of the pregnancy has also increased. A few examples are timing of repeat cesarean section, diagnostic amniocentesis, delivery of the diabetic mother, and intrauterine transfusion for Rh disease.

In recent years new technologies have contributed much to gestational dating. The accuracy of ultrasonography in determination of EGA has been widely studied and ultrasound is finding an increasing role in obstetrics.<sup>1</sup> Some authors have suggested that routine ultrasonography is the best method for establishing the EDC in all patients.<sup>2</sup> Laboratory analysis of amniotic fluid constituents, particularly phospholipids, assists in the evaluation of fetal maturity<sup>3</sup> but contributes little to pinpointing the EDC.

Despite technologic innovations, historical information and clinical examination during pregnancy remain the major methods of dating gestation in most obstetric practices. Rules for gestational dating are generally known, but there have been few studies of their real

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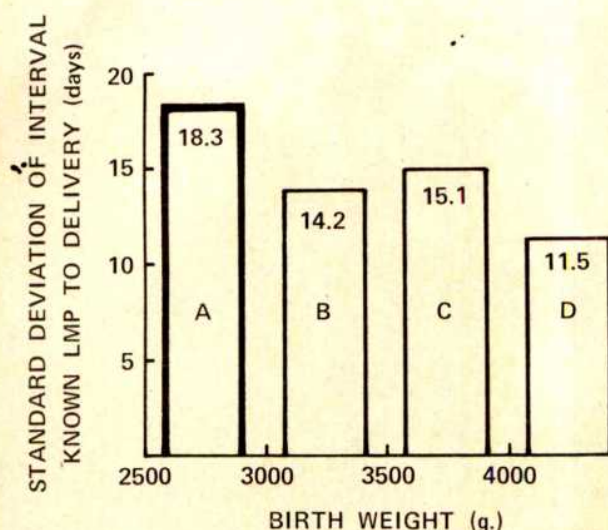


Fig. 1. Standard deviation of interval of known LMP to delivery (days). Group A (2,500 to 3,000 gm) is significantly larger than Groups B, C, and D ( $\geq 3,000$  gm) ( $p = 0.01$ ) by F test. No significant difference among Groups B, C, and D.

Table I. Descriptive data on patients delivered of infants weighing  $\geq 3,000$  gm

| Parameter                      | No.             |
|--------------------------------|-----------------|
| N                              | 418             |
| Age (yr)                       | $22 \pm 4.9^*$  |
| Gravidity                      | 2.2             |
| Parity                         | 0.8             |
| Weight (kg)                    | $66 \pm 15$     |
| (first examination)            |                 |
| Estimated gestational age (wk) | $18 \pm 8.0$    |
| (first examination)            |                 |
| No. of estimators              | $7.1 \pm 2.3$   |
| Infant weight (gm)             | $3,540 \pm 380$ |

\* $\pm$ S.D.

accuracy.<sup>4</sup> The purpose of this study is to examine estimators of gestational age and compare their relative ability to predict the date of delivery. Estimators chosen for evaluation were last menstrual period (LMP), quickening (QIK), fetal heart tones audible with an unamplified fetoscope (FHT), uterus at the umbilicus (UMB), and fundal height (FH). Future studies will examine the value of combining these estimators in an effort to improve the accuracy of gestational dating.

#### Material and methods

Sequential obstetric charts of patients seen in the resident clinics at the University of Michigan's Women's Hospital and Wayne County General Hospital during randomly selected periods in 1978 and 1979 were reviewed and data were retrieved on each patient's age, weight, obstetric history, gestational age at first examination, infant weight, delivery date, gestational dating

parameters, and ultrasound examinations. The initial criterion for inclusion in the study was the spontaneous onset of labor resulting in the delivery of a normal singleton infant weighing  $\geq 2,500$  gm. All gestational estimators were dated with reference to delivery date (i.e., days from the event to delivery). Specific criteria for gestational estimators were established.

**LMP.** The LMP was defined as "known" if a specific date was given by the patient and she had not been using oral contraceptives during the cycle preceding the LMP. Uncertain responses were classified as "uncertain week" if the patient could say approximately when in the month the LMP occurred (e.g., "early May" or "mid-March"). In these cases the LMP was arbitrarily assigned the fifth, fifteenth, or twenty-fifth of the month for early, middle, and late responses. If only the month could be remembered, "uncertain month," the LMP was arbitrarily assigned as the fifteenth of that month.

**QIK.** The date when fetal movement was first appreciated by the patient, as recorded on the chart, was used if an exact date was given.

**FHT.** The date on which fetal heart tones were first audible with an unamplified fetoscope was used only if the absence of FHT was recorded within 2 weeks previously.

**UMB.** If the uterus was at the level of the umbilicus at examination, the date was recorded. If the uterus was 1 cm above or below the umbilicus, the date of the uterus at the umbilicus was arbitrarily assigned 1 week earlier or later accordingly.

**FH.** Fundal height in centimeters and the date were recorded for measurements from 18 to 36 cm. FH measurements were made from the symphysis pubis, "over the curve," to the top of the uterine fundus.

All data were analyzed with the use of the programs of the Statistical Research Laboratories of the University of Michigan on the Michigan Interactive Data Analysis System. Standard deviation and the ninetieth percentile of the absolute value of the deviation from the mean were used as the measures of precision of the gestational estimators. The ninetieth percentile of the absolute value of the deviation from the mean is the measured 90% tolerance level for this sample group. Comparisons of the standard deviations were made with the F test.\*

#### Results

A total of 535 patients were included in the study under the initial criteria. When the interval from occurrence of a gestational estimator to delivery was ex-

\*The F test examines equality of variances. The standard deviation is the positive square root of the variance.



**Table II.** LMP: Interval to delivery of an infant weighing  $\geq 3,000$  gm

|                      | N   | Mean  | S.D.  | Ninetieth percentile of abs. dev.* |
|----------------------|-----|-------|-------|------------------------------------|
| LMP known:           | 297 | 284.2 | 14.6† | 23.2                               |
| Primiparous patients | 153 | 283.5 | 11.3‡ | 16.5                               |
| Multiparous patients | 144 | 285.0 | 17.5‡ | 31.0                               |
| LMP uncertain:       |     |       |       |                                    |
| Uncertain week       | 83  | 286.7 | 23.8† | 42.7                               |
| Uncertain month      | 20  | 291.2 | 36.1† | 47.2                               |

All data in days unless otherwise noted.

\*Ninetieth percentile of the absolute value of the deviation from the mean.

†Difference between results for known LMP and uncertain LMP were significantly different ( $p < 0.001$ ) by F test.

‡Difference between results for primiparous and multiparous patients were significantly different ( $p < 0.001$ ) by F test.

aminated by 500-gram weight groups (e.g., Fig. 1), a significantly higher variation, as measured by standard deviation, was found for infants weighing  $< 3,000$  grams. Additionally, several infants in the group weighing 2,500 to 3,000 grams were clearly premature by examination and by outcome, including two infants who developed severe respiratory distress syndrome. There were no significant differences among the standard deviations of the estimators by weight groups for infants weighing  $\geq 3,000$  gm; therefore, this subgroup of 418 patients was used for the remaining analyses. Descriptive data for the 418 patients are presented in Table I. The studied gestational estimators, measured as days to delivery, were distributed in an approximately normal fashion.

In Table II the interval in days from LMP until delivery is listed. A specific date for LMP was given by 71% of the patients, 25% gave an approximate date, and 4% could not give any date for LMP. The mean intervals to delivery were not significantly different, but the standard deviations were significantly larger for patients with an uncertain LMP ( $p < 0.001$ ). When known LMP was examined for primiparous and multiparous patients, it was found that primiparous patients had a significantly smaller standard deviation of the interval from LMP to delivery than multiparous patients ( $p < 0.001$ ). The mean plus and minus the ninetieth percentile of the absolute value of the deviation from the mean describes the interval within which 90% of the patients were delivered. Thus, 90% of the patients with a known LMP were delivered within  $\pm 23$  days of the date predicted by the mean for known LMP (i.e., between 261 and 307 days after the known LMP).

Table III describes QIK, FHT, and UMB. Comparison of primiparous and multiparous patients revealed a statistically significant difference only for QIK. A

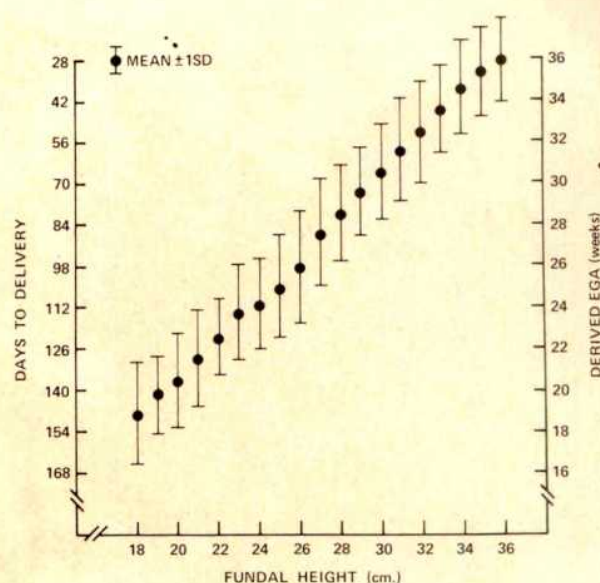


**Fig. 2.** Fundal height and days to delivery in 418 patients delivered of infants weighing  $\geq 3,000$  gm. "Derived" EGA was calculated by assuming the delivery date as day 280 of gestation and subtracting the mean days to delivery.

"derived EGA" was calculated for each estimator by assuming the delivery date to be day 280 of gestation and subtracting the mean interval for the estimator.

FH measurements in centimeters (from 18 to 36 cm) are presented in Table IV; each patient had an average of 4.3 FH measurements. The interval from any particular FH measurement to delivery did not differ significantly between primiparous and multiparous patients. Although there seems to be considerable variation in the precision of FH measurements, as assessed by standard deviation, no significant trends could be found. Gestational age was found to relate to FH in a linear fashion (Fig. 2), with a slope of 0.980.

Paired analysis of the gestational estimators was performed to evaluate the relative precision of each estimator. Known LMP was significantly more precise than FHT ( $P < 0.05$ ), FH ( $p < 0.05$ ), and QIK ( $p < 0.01$ ). UMB was better than QIK ( $p < 0.05$ ). FHT, FH, and QIK were not significantly different in terms of precision. On the basis of these results, the estimators were ordered according to decreasing precision as follows: (1) LMP, (2) UMB, and (3) FHT, FH, and QIK. The relative order of precision among UMB, FHT, FH, and QIK was not different for patients with known or uncertain LMP.

# **Comment**

The EDC is usually predicted from Naegle's rule in patients with an accurate LMP. This rule predicts an EDC 280 to 284 days from the LMP and its apparent accuracy has made it a standard part of obstetric care.



**Table III.** Obstetric landmarks: Interval to delivery of infant weighing  $\geq 3,000$  gm

|     | All patients |       |      |                                    |                   | Primiparous patients |        |      | Multiparous patients |        |      |
|-----|--------------|-------|------|------------------------------------|-------------------|----------------------|--------|------|----------------------|--------|------|
|     | N            | Mean  | SD   | Ninetieth percentile of abs. dev.* | Derived EGA† (wk) | N                    | Mean   | SD   | N                    | Mean   | SD   |
| QIK | 204          | 156.3 | 18.0 | 28.3                               | 17.7              | 119                  | 152.8‡ | 17.5 | 85                   | 161.4‡ | 17.6 |
| FHT | 182          | 136.2 | 17.0 | 26.8                               | 20.5              | 107                  | 136.1  | 15.8 | 75                   | 136.3  | 18.6 |
| UMB | 95           | 140.8 | 14.9 | 26.8                               | 19.9              | 67                   | 142.1  | 13.4 | 28                   | 137.5  | 17.9 |

All data in days unless otherwise noted.

\*Ninetieth percentile of the absolute value of the deviation from the mean.

†Derived EGA is gestational age in weeks calculated by assuming delivery date to be day 280 of gestation and subtracting mean interval to delivery.

‡Difference between results for primiparous and multiparous patients were significant ( $p < 0.01$ ) by F test.

**Table IV.** FH measurements: Interval to delivery of an infant weighing  $\geq 3,000$  gm

| FH (cm) | N   | Mean  | SD   | Ninetieth percentile of abs. dev.* | Derived EGA† (wk) |
|---------|-----|-------|------|------------------------------------|-------------------|
| 18      | 61  | 148.2 | 17.3 | 22.9                               | 18.8              |
| 19      | 52  | 142.4 | 13.4 | 24.4                               | 19.7              |
| 20      | 76  | 138.1 | 15.9 | 25.9                               | 20.3              |
| 21      | 58  | 130.0 | 15.8 | 24.0                               | 21.4              |
| 22      | 53  | 123.1 | 13.2 | 19.1                               | 22.4              |
| 23      | 58  | 114.7 | 16.4 | 28.3                               | 23.6              |
| 24      | 87  | 112.0 | 15.3 | 23.0                               | 24.0              |
| 25      | 67  | 105.7 | 16.6 | 28.3                               | 24.9              |
| 26      | 89  | 99.2  | 19.3 | 29.8                               | 25.8              |
| 27      | 84  | 88.4  | 17.5 | 29.6                               | 27.4              |
| 28      | 86  | 81.7  | 15.6 | 26.3                               | 28.3              |
| 29      | 78  | 74.5  | 15.0 | 26.5                               | 29.4              |
| 30      | 121 | 68.1  | 15.6 | 24.1                               | 30.3              |
| 31      | 77  | 61.0  | 16.8 | 27.0                               | 31.3              |
| 32      | 131 | 53.7  | 17.2 | 27.7                               | 32.3              |
| 33      | 131 | 47.2  | 15.4 | 25.8                               | 33.3              |
| 34      | 144 | 39.3  | 15.6 | 25.7                               | 34.4              |
| 35      | 136 | 33.4  | 15.0 | 23.4                               | 35.2              |
| 36      | 96  | 28.4  | 13.9 | 23.4                               | 35.9              |

All data are in days unless otherwise noted.

\*Ninetieth percentile of the absolute value of the deviation from the mean.

†Derived EGA is gestational age in weeks calculated by assuming delivery date to be day 280 of gestation and subtracting mean interval to delivery.

There are several studies to document the gestational period in humans and most have concluded that it is approximately 278 to 284 days with a standard deviation of 8 to 15 days.<sup>5, 6</sup> Other estimators of gestational age, however, are much less well studied. Two studies have estimated quickening to occur at 19 to 20 weeks in primiparous women and at 17 to 18 weeks in multiparous women with a standard deviation of 15 days.<sup>7</sup> Although the use of FHT and UMB is mentioned in obstetric texts,<sup>8, 9</sup> no references are cited, and a review of the literature revealed only two recent studies on

small groups of patients.<sup>10, 11</sup> FH has been studied as a predictor of growth retardation and a close correlation with gestational age has been found.<sup>12, 13</sup>

Because of the difficulties with clinical evaluation of gestational age, some authors have recommended that ultrasound be used routinely to date pregnancies.<sup>2</sup> Accuracy as good as  $\pm 3$  days (95% confidence limits) has been claimed for some ultrasound techniques involving early examination or multiple studies.<sup>1</sup> However, another study found the ultrasound-predicted date of delivery to be less accurate ( $\pm 12$  days), presumably because of inherent biologic variability.<sup>2</sup> It is not clear that routine ultrasonography of all pregnant patients is practically possible or necessary. It is important that the maximum information available from clinical examinations be evaluated to help determine which patients can benefit from ultrasonography.

Most studies of the clinical evaluation of gestational age have examined the occurrence of an event in relation to the LMP, which has been shown to have a range of error in predicting the date of delivery. Results of Dubowitz examinations of infants have also been used as reference points, but Dubowitz scoring data are based upon interval from LMP.<sup>14</sup> In this study we attempted to examine LMP in the same light as other gestational estimators and thus use as a reference point the delivery date of a "term" infant, which can be easily defined and is not based on one of the estimators under study.

In the absence of other criteria, weight  $\geq 2,500$  gm has been used to define maturity; however, we found that the group weighing 2,500 to 3,000 gm included some clearly premature infants, which was also reflected in the statistically larger variability in delivery date relative to the occurrence of gestational estimators. The criterion of weight  $\geq 3,000$  gm was used with the intent of selecting a relatively homogenous group of normal, term infants for study. From this point it may



be possible to examine deviations in abnormal populations such as growth-retarded or premature infants.

The data on gestational estimators in this study generally agree with those of previous studies, with the exception of a smaller difference between quickening in primiparous and multiparous patients. LMP is the most precise predictor of EDC. This may be due in part to the fact that it is an easily recognized event for which a specific date can be determined. Parity seems to have little effect on the time of the occurrence of the estimators, with the exception of quickening, but does influence precision, particularly of LMP. This can be of importance when one is faced with conflicting estimates of EDC in a patient. Although some authors have suggested that FH is of little value in estimating gestational age,<sup>15</sup> in the present study we found that in normal pregnancy FH may be better than quickening and perhaps as useful as FHT in the estimation of the EDC.

The data presented here suggest that the EDC can be predicted with 90% certainty only within  $\pm 3$  weeks by even the best single estimator. This corresponds closely with the findings of Hertz and associates<sup>4</sup> that the estimated gestational age by a single estimator must be 42 weeks in order to be 90% certain that the patient is at least at 38 weeks' gestation. Several factors may contribute to the large error apparently inherent in clinical dating of pregnancy: In this study the patients were examined by multiple physicians; and the data were collected retrospectively; therefore, there was no pos-

sibility of controlling techniques by which gestational estimators were obtained. Jimenez and associates<sup>10</sup> recently reported a high degree of precision in recording gestational estimators in a small group of patients seen by one examiner.<sup>10</sup> However, it is not certain that this precision could be duplicated in a large number of patients in a practice setting involving several physicians.

In the course of prenatal care, particularly when the patient is seen early in pregnancy, it is possible to obtain several measures of gestational age and it has been suggested that combination of these estimators may improve the accuracy of prediction of EDC.<sup>16</sup> To date we are not aware of any studies of the value of combining multiple estimates of gestational age to improve the accuracy of predicting the EDC. It must also be noted that clinical estimators of gestational age are, with the exception of LMP, indirect measurements of the size of the fetus and uterine contents. Multiple external factors, such as the patient's weight, height, position, full or empty bladder, etc., may influence the measurements. Additionally, examiner-specific factors such as auditory acuity may play a role. Therefore, it would be valuable to obtain data from each individual examiner in order to correlate all measurements with actual gestational age by some well-defined criteria. In the absence of such information, the present data provide guidelines as to the degree of confidence that can be placed on estimates of the gestational age based on clinical examination and history.

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# The effect of diazepam on rates of fertilization in the CF-1 mouse

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Diazepam in a dose of 0.75, 1.25, or 2.5 mg was force-fed to mice during 3 days of oocyte maturation and superovulation induced with pregnant mare's serum and human chorionic gonadotropin (hCG). Twenty-two hours after hCG was administered, rates of fertilization were determined in squash preparations. Increasing doses of diazepam were associated with increasing numbers of degenerated eggs and decreasing rates of fertilization when compared to results in control animals. Chromosome preparations of ova from treated animals confirmed the chromatin degeneracy and failed to document an arrest of maturation of the ova. Correlative serum levels of diazepam and desmethyldiazepam were measured in blood obtained 2 hours after the indicated dose. (AM. J. OBSTET. GYNECOL. 139:178, 1981.)

DIAZEPAM is an agent much used for a number of purposes, but mainly for its tranquilizing properties. The pharmacokinetics of this agent has been well studied and reviewed.<sup>1, 1a, 2</sup> The presence of free, unbound diazepam in the plasma is about 2% to 3% of the total amount of drug present and is approximately equivalent to the amount of its major metabolite desmethyldiazepam.<sup>3</sup> It has been shown that diazepam in vitro causes meiotic arrest of ova from virgin female CF-1 mice in culture. With increasing concentrations of up to 50  $\mu\text{g/ml}$  an increasing degree of arrest at metaphase I was seen, and higher levels of up to 200  $\mu\text{g/ml}$  caused an increasing proportion of degenerated ova, but no aneuploidy was detected.<sup>4</sup> The present study was undertaken to determine the effects of diazepam on rates of fertilization in CF-1 mice in vivo. Karyotypic preparations from control and treated mice were also obtained as were serum levels of diazepam and desmethyldiazepam.

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## Methods and material

Oocytes were collected from treated and control virgin CF-1 mice (Charles River) following superovulation<sup>5</sup> with an intraperitoneal injection of 10 U of pregnant mare's serum (PMS) followed in 44 to 46 hours by 5 U of human chorionic gonadotropin (hCG). The treated animals were force-fed either 0.75, 1.25, or 2.5 mg of diazepam each morning for 3 days, beginning the morning of PMS administration. The upper limit of this dosage regimen is that which in a comparable dose by weight decreased litter size and the number of surviving neonates in rats.\* Two control animals were studied concurrently with the experimental group. The diazepam was weighed to the dose indicated and placed into the end of a 14-gauge needle with the tip ground flat. The needle guard served as a plunger to effect deep intrapharyngeal placement of the drug. The third dose of diazepam was administered simultaneously with the hCG injection. The treated and control animals (no diazepam) were then placed one to a cage with 20- to 30-week-old CF-1 male mice for 6 hours. Those who had mated as evidenced by the presence of a vaginal plug were killed 22 hours after hCG by cervical dislocation. The fallopian tubes were isolated and the eggs were placed in Krebs-Ringer medium with hyaluronidase by puncturing the ampulla under a dissecting microscope. The eggs were transferred into two successive dishes of fresh Krebs-Ringer

\*Studies performed by Roche Labs.



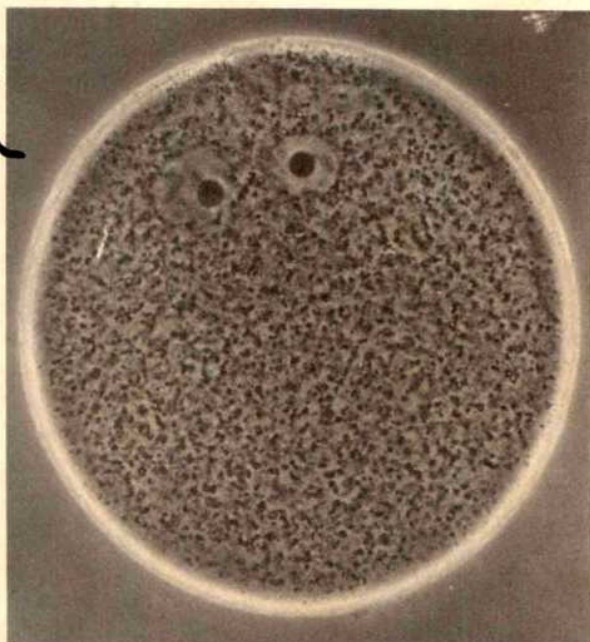


**Fig. 1.** Squash preparation of control ovum showing male and female pronuclei.

medium. Following this last transfer, fertilization rates of the eggs which did not appear degenerated under the dissecting microscope were assessed. Approximately 10 eggs were placed in a drop of medium on a clean slide and pressed lightly between a coverslip and the slide after a small amount of a petroleum jelly-paraffin mixture was placed at the four corners of the coverslip. Fertilization was judged to have occurred when two pronuclei (male and female) could be visualized in the squash preparation under phase contrast microscopy (Fig. 1).

In an attempt to determine if the higher doses of oral diazepam caused an arrest at metaphase I or metaphase II, eggs from an additional 12 superovulated animals with vaginal plugs, four control animals, and four animals treated in the manner described with 1.25 and 2.5 mg of diazepam respectively, were submitted to analysis of a fixed and stained chromosomal preparation. This was done by modification of the air-drying method of Tarkowski.<sup>6</sup>

In a third part of this study, levels of diazepam and desmethyldiazepam were measured with the use of pooled sera\* from five control animals and groups of five animals given 0.75, 1.25, and 2.5 mg of diazepam,

\*Obtained by means of high-performance lipid chromatography, Harborview Medical Center Toxicology Laboratory, Seattle, Washington.

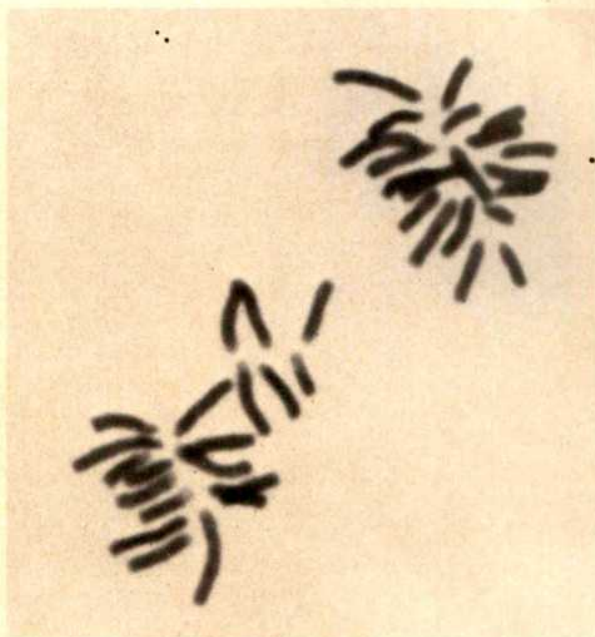


**Fig. 2.** Chromosome spread of ovum of animal given 1.25 mg of diazepam. Normal fertilized haploid complements.

respectively. Blood was obtained by retroorbital puncture in ether-anesthetized animals 2 hours following the oral dose, the time of peak blood levels in man following oral administration and a time of maximal sedation observed in mice.

### Results

As seen in Table I, the mating frequency was somewhat decreased, although not significantly, at dosage levels of 1.25 and 2.5 mg, but the average number of eggs per animal was approximately the same, implying that these levels of the drug do not block superovulation. The percentage of degeneracy as seen grossly under the dissecting microscope was significantly increased with an increasing dosage of the drug.

Table II shows the trend toward decreasing rates of fertilization of the grossly nondegenerated eggs with increasing doses of diazepam. A significant difference with respect to the controls was not obtained with the 0.75 mg dose but was noted at 1.25 mg and 2.5 mg.

The chromosomal study revealed two haploid sets of chromatin from both control and experimental groups in a frequency range that would be predicted from the squash preparations. In intact spreads no aneuploidy was detected (Fig. 2). The remainder of the preparations showed degenerated or fragmented chromosomal material. No metaphase I or metaphase II stages were seen. It is assumed that those eggs with degenerat-



**Table I.** Mating frequency, egg production, and incidence of degenerated eggs as seen grossly under dissecting microscope

|         | Mated females/total | Average No. of eggs/animal | Total No. of eggs | Degenerate* (%) |
|---------|---------------------|----------------------------|-------------------|-----------------|
| Control | 7/16                | 34                         | 238               | 13.4            |
| 0.75 mg | 6/17                | 31                         | 186               | 15.6            |
| 1.25 mg | 6/20                | 27                         | 162               | 24.1†           |
| 2.5 mg  | 5/21                | 33                         | 165               | 28.3†           |

\* $\chi^2_{(1)} = 17.15$ ;  $p < 0.001$ . . . a trend exists for increasing rates of degenerated eggs with increasing dosages of diazepam.

†Significantly different from controls by chi-square test ( $p < 0.01$ ).

**Table II.** Rates of fertilization in grossly nondegenerated eggs

|          | Eggs fertilized |      | Eggs not fertilized* |      |
|----------|-----------------|------|----------------------|------|
|          | No.             | %    | No.                  | %    |
| Control  | 140             | 67.9 | 66                   | 32.1 |
| 0.75 mg  | 101             | 64.3 | 56                   | 35.7 |
| 1.25 mg† | 68              | 55.3 | 55                   | 44.7 |
| 2.5 mg†  | 46              | 39   | 72                   | 61   |

\* $\chi^2_{(1)} = 26.08$ ;  $p < 0.01$ . . . a trend exists for decreasing rates of fertilization with increasing dosages of diazepam.

†Significantly different from controls and other dose levels by chi-square test ( $p < 0.01$ ).

ing chromatin but not gross cytoplasmic degeneration under the dissecting microscope would not participate in fertilization leading to the formation of two discrete pronuclei.

Those animals without vaginal plugs were randomly sampled and they had approximately as many tubal eggs as those who had mated. Thus, mating per se did not induce superovulation.

The extremely elevated serum levels of the major metabolite of diazepam, desmethyldiazepam, a compound with psychoactive properties similar to those of the parent compound,<sup>1-1a</sup> are shown in Table III. Apparently rapid metabolism of diazepam to desmethyldiazepam had occurred, and had blood been obtained earlier after oral treatment, the serum levels of diazepam presumably would have been higher.

### Comment

Edwards and Gates<sup>7</sup> demonstrated that exogenous gonadotropins produce stages of meiosis, ovulation, and fertilization which closely approximate the natural sequence. This has provided a means of obtaining large numbers of eggs to study the effects of compounds that have been shown to inhibit either ovulation or meiotic maturation.<sup>8</sup>

The control data obtained in the present experiment are similar to those observed by Donahue<sup>9</sup> using the same experimental protocol. He did observe a some-

**Table III.** Serum levels of diazepam and its major metabolite 2 hours after oral treatment

|         | Diazepam ( $\mu\text{g/ml}$ ) | Desmethyldiazepam ( $\mu\text{g/ml}$ ) |
|---------|-------------------------------|----------------------------------------|
| Control | Negative                      | Negative                               |
| 0.75 mg | 0.30                          | 6.80                                   |
| 1.25 mg | 1.30                          | 10.80                                  |
| 2.5 mg  | 1.20                          | 14.20                                  |

what higher rate of fertilization at 22 hours after hCG, with 77% of eggs in the pronuclear stage. He further found that only one of 175 eggs not fertilized was in metaphase II. All others not fertilized mostly showed fragmented or degenerate chromatin. Three percent revealed a configuration which was not definable. The average number of eggs appearing grossly degenerated in his series was approximately that obtained in both our control animals and those receiving 0.75 mg of diazepam.

In the present study, diazepam did not inhibit ovulation, an effect seen in mice with many other tranquilizers. Chlorpromazine and thioridazine completely block superovulation in high doses, but chlordiazepoxide, one of the benzodiazepine class of drugs, only partially inhibits superovulation at a dose of 4 mg per animal.<sup>10</sup> Meprobamate, an agent which has been shown to concentrate in ovarian follicular fluid<sup>11</sup> and which can block ovulation in mice,<sup>10, 12</sup> seems to arrest meiosis in vitro at higher concentrations, but this effect does not occur in vivo.<sup>13</sup> Diazepam did seem to decrease mating frequency at the higher doses, an effect previously observed and attributed to the behavioral effects of the drug.<sup>14</sup>

In terms of the possible mechanism of action of diazepam on meiosis in the mouse, it is of interest to note that diazepam inhibits the synthesis and accumulation of the myosin heavy chain in cultures of chick embryo myoblasts, which arrests normal muscle cell differentiation.<sup>15</sup> Therefore, it is possible that diazepam may interfere with meiosis by blocking protein synthesis. A similar effect has been postulated for puromycin, a



protein-synthesis inhibitor that interferes with meiosis by its general effect on protein synthesis<sup>8</sup> as well as by preventing formation of the spindle apparatus.<sup>16</sup>

The serum levels obtained in this study are quite high but within the range seen in man following long-term large doses.<sup>17</sup> Diazepam is lipid soluble and water insoluble. After oral ingestion and gastrointestinal absorption, there is extensive tissue distribution of the parent compound. In man, metabolism proceeds slowly, with a half life between 20 and 50 hours.<sup>1</sup> The results obtained with diazepam *in vivo* when the drug is given for the 3 days of oocyte maturation and ovulation reveal the same trend toward dose-related chromatin degeneration as had been seen *in vitro*.<sup>4</sup> This results in

decreased rates of fertilization, as seen in our *in vivo* experiments. It is known in humans, because of the slow metabolism of the drug, that accumulation can occur, particularly if the drug is given up to three times daily. This accumulation could conceivably affect the meiotic process. Therefore, although evidence of teratogenicity for diazepam is lacking, our results suggest that subfertility could be a consequence of long-term and/or high-dose administration.

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# Effects of vasoactive polypeptides on the uterine vasculature

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Estrogen-induced increases in uterine blood flow appear to require de novo protein or polypeptide synthesis. In the present experiments a chronically catheterized nonpregnant sheep preparation was used to determine the uterine vascular effects of vasoactive intestinal polypeptide (VIP), neurotensin, and substance P. These effects were compared to those of bradykinin and the most potent vasodilator prostaglandin, prostacyclin. An intra-arterial catheter was placed in a branch of the main uterine artery to allow administration of the compounds directly into the uterine vasculature. Uterine blood flow was continuously monitored via an electromagnetic flow transducer on the main uterine arteries. VIP, bradykinin, and prostacyclin were equally potent as vasodilators of the uterine vasculature, while neurotensin and substance P were totally devoid of vasoactivity. Unlike estradiol, bradykinin and VIP produced significant changes in systemic arterial pressure and heart rate, suggesting that these compounds may not be responsible for mediating the uterine vascular response observed after estrogen. However, VIP was a potent uterine vasodilator and was able to totally ablate uterine contractile activity, suggesting that this endogenously occurring polypeptide may be important in regulating uterine hemodynamics and contractile activity. (AM. J. OBSTET. GYNECOL. 139:182, 1981.)

ESTROGEN has been shown to induce a profound dilation of the uterine vasculature.<sup>1, 2</sup> This increase in uterine blood flow begins 30 to 40 minutes after the administration of estrogen<sup>1</sup> (see Fig. 2). In addition, the uterine vascular response to estrogen can be blocked by a constant infusion of cycloheximide, a protein synthesis inhibitor,<sup>1</sup> begun shortly before the estrogen stimulus. These data strongly suggest that estrogen does not

act directly to cause uterine vasodilation but instead leads to generation of a new protein (i.e., enzyme) or polypeptide which effects the uterine vascular response. Surprisingly, attempts to define the mechanisms of the estrogen response have included studies of only one vasoactive polypeptide, bradykinin, on the uterine vasculature.<sup>2</sup>

In recent years, several new vasoactive polypeptides have been isolated and purified by investigators of neurophysiology and gastroenterology. One such peptide is vasoactive intestinal polypeptide (VIP), which was originally isolated in the small intestines of dogs by Said and Mutt, in 1970.<sup>3</sup> Since its original localization, VIP has been identified in several other tissues including the central nervous system,<sup>4</sup> pancreas,<sup>5</sup> female genitourinary system,<sup>6, 7</sup> and placenta.<sup>8, 9</sup> In the genitourinary system, VIP-containing nerves have been found to be intimately associated with vascular and uterine smooth muscle in several animal species (e.g., pigs, cats, rats, and mice)<sup>6</sup> and more recently in humans.<sup>7</sup>

VIP-containing nerves are found in uterine vascular smooth muscle between the adventitia and the media, suggesting vascular innervation.<sup>6</sup> In addition, the

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human placenta contains extremely high concentrations of VIP<sup>8</sup> and is also capable of synthesizing this compound.<sup>9</sup> VIP is a potent vasodilator in the small intestine,<sup>3</sup> but little information exists regarding its effects on other vascular beds. However, the physiologic role of this vasodilatory polypeptide could be important in both estrogen-induced vasodilation and regulation of uterine and placental hemodynamics during pregnancy.

Two other polypeptides, substance P and neurotensin, have also been identified in similar peptidergic nerves in both the central nervous system and the small intestine.<sup>10, 11</sup> Like VIP, substance P is an extremely potent vasodilator of a number of vascular beds,<sup>12</sup> while the vascular actions of neurotensin are less clearly defined.<sup>11</sup> The effects of substance P or neurotensin have not been described for the uterine vasculature.

Thus, the present experiments were designed to determine the effects of the vasoactive polypeptides VIP, substance P, and neurotensin on the uterine vasculature of the nonpregnant ewe and to compare these compounds to the potent uterine vasodilators bradykinin<sup>2</sup> and prostacyclin (prostaglandin I<sub>2</sub> [PGI<sub>2</sub>]).<sup>13</sup> In addition, these experiments were designed to determine if these vasoactive polypeptides were capable of producing vasodilation equivalent to that seen after the administration of estradiol-17 $\beta$ .

#### Material and methods

Six nonpregnant ewes of mixed breeds weighing between 40 and 60 kg were sedated with Valium (10 mg intramuscularly) and placed in the left lateral recumbent position. Following infiltration with Xylocaine (2% solution), the carotid artery and jugular vein were dissected free and cannulated with a polyvinyl catheter threaded proximally to the level of the aortic root and superior vena cava, respectively.

With the use of anesthesia (250 mg of thiopental and 12 mg Pontocaine Hydrochloride as a hyperbaric spinal), the uterus was exposed by a midline abdominal incision 15 to 20 cm in length and electromagnetic flow probes were placed on both uterine arteries (Fig. 1). To allow direct intra-arterial administration of compounds, the lateral branch of each uterine artery was catheterized distal to the flow probe. Catheters were filled with heparin (1,000 U/ml) to maintain patency and all animals were ovariectomized. Following operation, animals were placed in portable cages and were kept in the laboratory for the duration of the experiments. Ewes received water and commercial feed ad libitum. Each ewe received an intravenous injection of estradiol-17 $\beta$  (1  $\mu$ g/kg) each evening to prevent

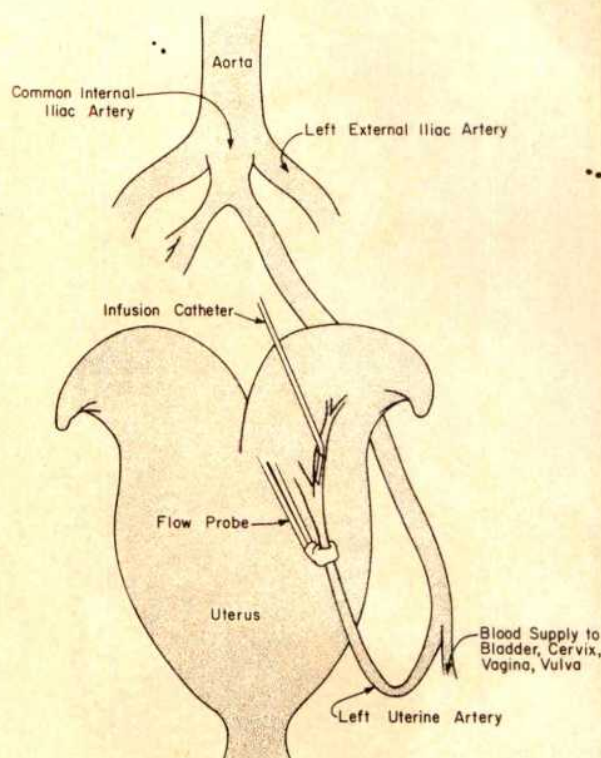


**Fig. 1.** Schematic diagram of the uterine vasculature showing the locations of the infusion catheter and the electromagnetic flow probe.

uterine atrophy which would occur because of castration. Uterine blood flow returned to the baseline value prior to testing on the following day.

Experiments were not performed before the fifth postoperative day to assure full recovery from stress. During all experiments uterine arterial blood flow was continuously monitored with a square-wave electromagnetic flowmeter (Dienco RF-1000, Los Angeles, California), and systemic arterial and intrauterine pressures (intraluminal balloon-tipped catheter) were measured with pressure transducers (Micron Instrument Company, Los Angeles, California). Heart rate was determined with a cardiometer (Beckman Instrument Company, Fullerton, California). All parameters were continuously recorded with a pen writing recorder (Beckman Dynograph R-612, Beckman Instrument Company).

On each day of testing, animals received only one compound which was administered as a 5-minute intra-arterial infusion. All doses were given in a randomized order and all parameters were allowed to return to baseline prior to the administration of a second dose. Systemic responses were recorded as the percent change from baseline values; uterine flow responses were measured as the maximum change in uterine



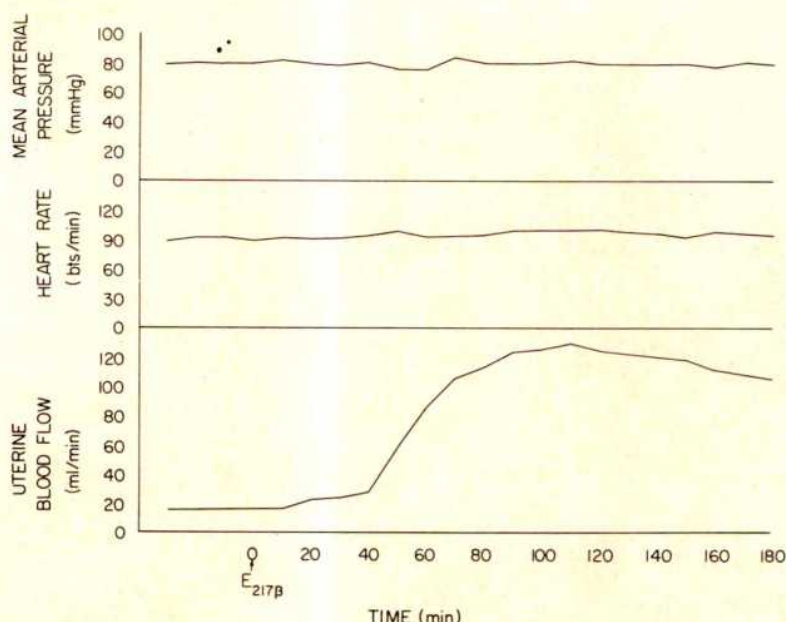


Fig. 2. Effect of a bolus injection of 1  $\mu\text{g/kg}$  of estradiol-17 $\beta$  on mean arterial blood pressure (*upper panel*), heart rate (*middle panel*), and uterine blood flow (*lower panel*), versus time after administration.

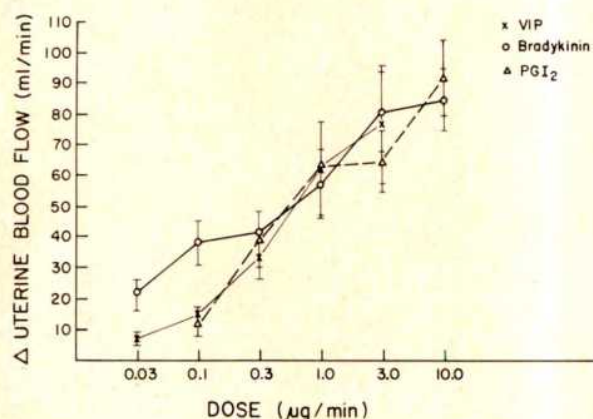


Fig. 3. Dose-response curve for 5-minute intra-arterial infusions of VIP, bradykinin, and prostacyclin ( $\text{PGI}_2$ ) in micrograms per minute versus changes in uterine blood flow in milliliters per minute.

blood flow at each dose. Each compound was tested in five animals.

Solutions of VIP, neurotensin, substance P, and bradykinin triacetate (Sigma Chemical Company, St. Louis, Missouri) were prepared fresh daily in saline. Prostacyclin sodium salt was prepared fresh daily in 0.01M Tris buffer, pH 9.4, to prevent degradation. Solutions for intra-arterial infusions were made up in 1.0 and 10  $\mu\text{g/ml}$  solutions and infused at the rate of 0.1, 0.3 or 1.0 ml/min, depending on the desired dose and its vasoactivity. Bolus doses of the compounds were also tested in a separate group of experiments. All

doses were expressed as the free base. Data were analyzed by means of Student's paired t test as described by Steel and Torrie.<sup>14</sup> The level of significance was  $P < 0.05$ .

## Results

**Typical estrogen response.** Estradiol-17 $\beta$  produces a profound vasodilation of the uterine vasculature without altering either arterial blood pressure or heart rate (Fig. 2). The increase in uterine blood flow occurs after a delay of 30 to 40 minutes. Maximum uterine vasodilation occurs at 90 to 110 minutes after administration of the steroid and returns to baseline in 5 to 6 hours. In the present study, intravenous injection of 1  $\mu\text{g/kg}$  of estradiol-17 $\beta$  given each evening produced a maximal increase in uterine blood flow with a mean  $\pm$  SEM of  $129 \pm 15$  ml/min for the six animals used in these studies.

**Effect of local intra-arterial infusions of polypeptides on uterine blood flow.** Local intra-arterial infusions of VIP, bradykinin, and the potent vasodilator  $\text{PGI}_2$  resulted in significant dose-related increases in uterine blood flow (Fig. 3). The recorded blood flow response for each dose was determined as the maximal sustained blood flow which was obtained during the 5-minute intra-arterial infusion. All three compounds shown in Fig. 3 appeared equally potent as uterine vasodilators in this preparation. In contrast, substance P and neurotensin were totally devoid of vasoactive properties in the uterine vasculature of the nonpreg-



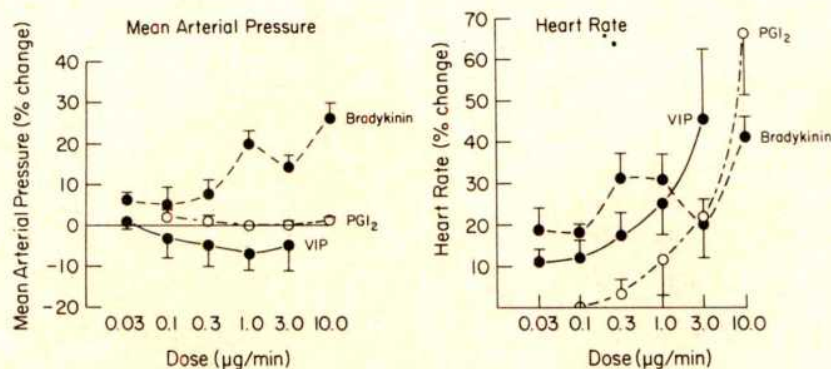


Fig. 4. Effect of 5-minute uterine artery infusions of VIP, bradykinin, and prostacyclin (PGI<sub>2</sub>) on mean arterial blood pressure (left panel) and heart rate (right panel). Values are the percentages of change from control.

nant ewe, even when infused in doses as high as 100 µg/min.

**Effect of intra-arterial infusions of polypeptides on systemic arterial blood pressure and heart rate.** Infusions of VIP produced a nonsignificant decrease in systemic arterial blood pressure (Fig. 4). In contrast, prostacyclin failed to modify while bradykinin significantly increased systemic arterial blood pressure. Substance P and neurotensin were without effect on systemic arterial blood pressure in the nonpregnant ewe.

Since VIP and bradykinin altered systemic arterial blood pressure, some of their effects on uterine blood flow may be due to changes in arterial blood pressure. To determine if these compounds produce vasodilation of the uterine vasculature, uterine vascular resistance was determined for each compound tested (Table I). Infusions of all three compounds resulted in a dose-related decrease in calculated uterine vascular resistance.

All three uterine vasodilator compounds (bradykinin, VIP, and PGI<sub>2</sub>) led to a dose-related increase in heart rate (Fig. 4, right side). Substance P and neurotensin did not alter systemic parameters at the doses tested.

**Effect of intra-arterial infusions of polypeptides on uterine contractile activity.** Intra-arterial infusions of VIP at the highest dose tested (3 µg/min) led to complete blockade of spontaneous contractile activity and total relaxation of uterine tone in the nonpregnant sheep uterus. In contrast, bradykinin increased uterine tone in a dose-related fashion but did not alter uterine contractile activity. Prostacyclin, neurotensin, and substance P did not significantly alter either contractile activity or uterine tone in this preparation.

**Duration of polypeptide-induced increases in uterine blood flow.** Table II shows the length of time required for uterine blood flow to return to baseline after

intra-arterial infusions of vasodilator polypeptides and prostacyclin were stopped. Following termination of the infusion, all three compounds (bradykinin, VIP, and PGI<sub>2</sub>) required a substantial amount of time (10 to 15 minutes) to return to baseline blood flow, suggesting that these vasoactive compounds would be able to sustain vasodilation even if not released at a constant rate.

**Comparison of intra-arterial infusions of polypeptides to bolus injections.** In the present study the effects of intra-arterial bolus injections of the polypeptides were also investigated. Bolus injections of vasoactive polypeptides and prostacyclin or constant infusions of the same compounds produced nearly identical local uterine and systemic responses, with the exception that equivalent vasodilator responses following bolus injections were obtained with doses approximately 10 times those used in the infusions. Since the data were virtually identical to those reported for infusions, the bolus injections data will not be presented.

**Bradykinin-induced increases in systemic arterial blood pressure and heart rate.** Because bradykinin is known to be a potent vasodilator, it was surprising to find that this peptide elevated systemic blood pressure in the unanesthetized sheep. In addition, this elevation in systemic arterial blood pressure might well have led to activation of baroreceptor reflex and subsequently to bradycardia, but instead an increased heart rate was observed. These observations were consistent with a bradykinin-induced release of an alpha- and beta-adrenergic receptor stimulant such as epinephrine.

To investigate the mechanism by which blood pressure and heart rate were elevated, the following experiment was undertaken in three nonpregnant ewes. Systemic responses to bolus intra-arterial injections of bradykinin were obtained, as shown in Fig. 5. Bradykinin produced increases in both blood pressure and heart rate. Responses to bradykinin were repeated



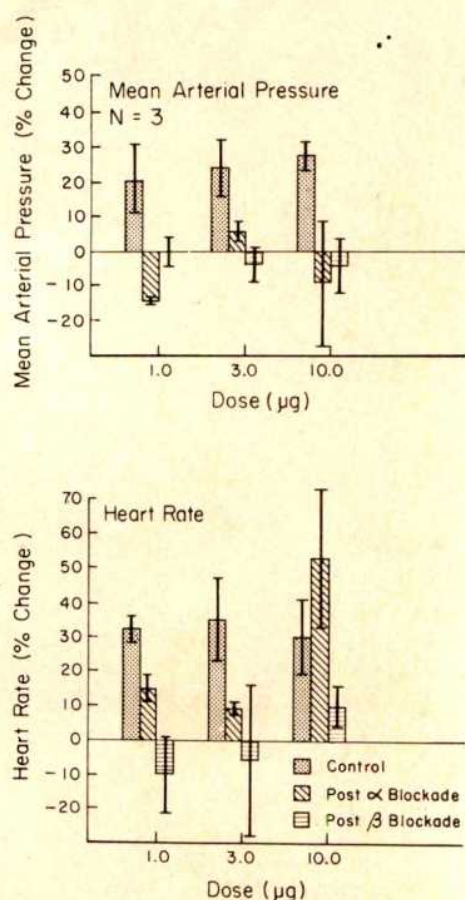


**Fig. 5.** Effect of uterine artery bolus injections of bradykinin on systemic arterial pressure (upper panel) and heart rate (lower panel) during the control period, after alpha-adrenergic blockade with phenoxybenzamine, and after beta-adrenergic-receptor blockade with propranolol.

after the systemic administration of 5 mg/kg of the alpha-adrenergic-blocking agent phenoxybenzamine, a dose which reduced systemic pressure responses to norepinephrine (10 to 30 μg) from  $28 \pm 4$  to  $2 \pm 4$  mm Hg. This dose of phenoxybenzamine completely blocked the bradykinin-induced increase in blood pressure and, at the lower doses (1.0 and 3 μg), significantly attenuated changes in heart rate. Subsequent blockade of beta receptors with propranolol (0.25 mg/kg) led to the complete abolishment of the observed tachycardia. Propranolol also completely ablated heart rate responses to 1 μg of isoproterenol ( $38 \pm 15$  to  $5 \pm 3$  bpm).

#### Comment

Numerous investigators have reported that estrogen dramatically increases uterine blood flow,<sup>1,2</sup> but to date the mechanism by which this steroid causes dilatation of the uterine vasculature has yet to be elucidated. Kil-

**Table I.** Calculated uterine vascular resistance

( $VR = \frac{BP}{BF}$ ) for 5-minute infusions ( $\pm$ SEM)

| Dosage<br>(μg/min) | Bradykinin<br>(mm Hg/<br>min/ml) | VIP<br>(mm Hg/<br>min/ml) | PGI <sub>2</sub><br>(mm Hg/<br>min/ml) |
|--------------------|----------------------------------|---------------------------|----------------------------------------|
| Baseline           | 5.9 ± 0.6                        | 7.8 ± 1.0                 | 7.7 ± 0.9                              |
| 0.03               | 2.6 ± 0.3                        | 4.9 ± 1.0                 | —                                      |
| 0.1                | 1.9 ± 0.3                        | 3.7 ± 0.5                 | 2.5 ± 0.9                              |
| 0.3                | 1.8 ± 0.2                        | 2.1 ± 0.4                 | 2.1 ± 0.6                              |
| 1.0                | 1.4 ± 0.2                        | 1.6 ± 0.4                 | 1.3 ± 0.3                              |
| 3.0                | 1.1 ± 0.2                        | 1.3 ± 0.4                 | 1.3 ± 0.2                              |
| 10.0               | 1.0 ± 0.1                        | —                         | 0.9 ± 0.1                              |

**Table II.** Duration of response after 5-minute infusion ( $\pm$ SEM)\*

| Dosage<br>(μg/min) | Bradykinin<br>(min) | VIP<br>(min) | PGI <sub>2</sub><br>(min) |
|--------------------|---------------------|--------------|---------------------------|
| 0.03               | 2.4 ± 0.7           | 1.8 ± 1.1    | —                         |
| 0.1                | 4.0 ± 0.4           | 4.0 ± 1.0    | 1.8 ± 0.6                 |
| 0.3                | 5.2 ± 0.4           | 6.2 ± 1.4    | 4.2 ± 1.1                 |
| 1.0                | 7.2 ± 0.9           | 10.3 ± 3.0   | 6.0 ± 1.1                 |
| 3.0                | 11.5 ± 1.0          | 13.4 ± 3.3   | 8.0 ± 0.7                 |
| 10.0               | 19.5 ± 7.0          | —            | 15.0 ± 1.7                |

\*Time from completion of infusion until uterine blood flow returned to baseline.

lam and co-workers<sup>1</sup> were the first to demonstrate that if an intra-arterial infusion of the protein synthesis inhibitor cycloheximide was begun shortly before an estrogen stimulus, it could completely block the estrogen blood flow response. This blockade was effective as long as the cycloheximide was infused continuously. Upon termination, estrogen-induced increases in uterine blood flow occurred after the normal time delay of 30 to 40 minutes. These data were interpreted to mean that de novo synthesis of a polypeptide or enzyme was required for the observed vasodilation. Recent studies in our laboratory<sup>15</sup> have confirmed and expanded the above report and have demonstrated that continuous protein (polypeptide) synthesis is required to maintain the estrogen-induced vasodilation. When cycloheximide is infused intra-arterially at the peak of the estrogen response, uterine blood flow remains constant for the first 10 to 20 minutes and then slowly decreases to approximately 60% of the peak uterine blood flow.<sup>15</sup> If the infusion is discontinued, uterine blood flow slowly returns to the previously observed peak level. These data suggest that continuous polypeptide or enzyme synthesis is required for the ongoing vasodilation. In addition, since cycloheximide infused at the peak is unable to completely obliterate the response to estrogen, it appears that the estrogen response may be mediated by more than one mechanism.



The present model has been used to evaluate the direct vascular effects of the intra-arterial infusion of several vasoactive polypeptides and prostacyclin. The investigation of uterine hemodynamics is in a precarious position in that there is no correct way of doing the experiment. If one does not castrate the animals, the sheep is subjected to a constantly changing hormonal milieu, while if the animal is castrated and a standardized hormonal treatment is used, an artificial hormonal milieu is created. No model is perfect, but the present model is at least constant in regard to the hormonal environment. A second important point regarding the method used in the current study, is whether the doses used are pharmacologic or physiologic. It is difficult to determine in which class these doses fall since very little is known about plasma and tissue levels of these polypeptides. To further complicate the procedure, it is impossible to determine the actual level of polypeptides at the site of action.

Our knowledge of the effects of polypeptides on the uterine vasculature is at present limited. To date, only angiotensin<sup>16</sup> and bradykinin<sup>2</sup> have been extensively investigated. The present work confirms and extends our knowledge about the effects of vasoactive polypeptides on the uterine vasculature and specifically investigates the vascular effects of VIP, substance P, neurotensin, and bradykinin. The vascular effects of these polypeptides were compared in the same animals to those of prostacyclin, the most potent uterine vasodilator prostaglandin.<sup>13</sup>

Substance P<sup>11</sup> and neurotensin<sup>12</sup> have been reported to be potent vasodilators in several different vascular beds. In the present study, both of these compounds failed to demonstrate any effects on the uterine vasculature. (This lack of activity was not due to the degradation of the compounds prior to administration since substance P in doses one thousandth [1/1,000] of those used in the current experiments resulted in a profound depressor response in anesthetized dogs. Similarly, neurotensin exhibited potent vasodepressor activity in the anesthetized rat.) The lack of vascular response in nonpregnant ewes suggests that receptors for these compounds do not exist in the ovine uterine circulation.

Bradykinin is a potent uterine vasodilator, as was previously reported.<sup>2</sup> In the current study, bradykinin infusions or bolus injections resulted in increases in systemic arterial blood pressure and heart rate. These systemic effects were totally abolished by alpha- and beta-adrenergic receptor blockade and appear to be mediated by a bradykinin-induced release of catecholamines. Feldberg and Lewis<sup>17</sup> have previously reported that bradykinin can release catecholamines from the adrenal, thus raising systemic blood pressure and heart

rate. It seems reasonable that if the estrogen-induced increases in uterine blood flow are mediated by the local uterine formation of bradykinin, then release of this compound into the circulation would lead to detectable changes in blood pressure or heart rate. However, several studies<sup>1, 2</sup> have shown that neither systemic arterial blood pressure nor heart rate changes occur during the uterine response to estrogen. A second possibility is that locally formed bradykinin might be degraded at the site of action, thus preventing any systemic effects. Thus, the proof as to whether or not this polypeptide involved in the estrogen response will have to await the use of a specific bradykinin antagonist and/or accurate analytical measurements.

VIP was found to be equipotent with prostacyclin and bradykinin as a vasodilator of the uterine vasculature. The doses used during intra-arterial infusions did not significantly change arterial blood pressure but did lead to significant increases in heart rate. This increased heart rate is most likely a compensatory response to peripheral vasodilation. Another interesting aspect of the VIP infusion was that VIP was able to abolish all spontaneous contractile activity in the nonpregnant ewe. The observation by Larsson and associates<sup>6</sup> that VIP was associated not only with vascular smooth muscle but also with the smooth muscle wall of the uterus suggests a possible role for VIP as a regulator of uterine contractile activity. These observations have been further substantiated by Ottesen and Wagner,<sup>18</sup> who have recently shown that VIP can totally abolish both oxytocin- and PGF<sub>2α</sub>-induced myometrial contractile activity in rabbits.

Recently others have reported that the human placenta not only contains large concentrations of VIP<sup>8</sup> but also is capable of synthesizing VIP.<sup>9</sup> The potential importance of the production of polypeptide in the uteroplacental circuit which can act as a local vasodilator and to prevent uterine contractile activity is obvious. This polypeptide could play a crucial role in maintaining both uterine blood flow and uterine quiescence during pregnancy.

The possibility that VIP plays a major role in estrogen-induced increases in uterine blood flow is more questionable. This compound, like bradykinin, did increase uterine blood flow significantly but exhibited systemic effects (increased heart rate) at doses which have only modest effects on uterine blood flow. In addition, preliminary experiments in our laboratory have shown that the uterus does not release VIP during the estrogen response since systemic arterial blood levels in the sheep are higher than uterine venous levels throughout the response.<sup>19</sup>

In summary, in the present study we investigated the



vasoactive properties of several polypeptides. Substance P and neurotensin appear to be totally devoid of uterine vascular activity, while bradykinin and VIP appear to be equipotent as vasodilators in the ovine uterine vasculature. These polypeptides exhibit vasodilation equivalent to that seen with prostacyclin, the most potent vasodilator prostaglandin. Most important, not only is VIP a uterine vasodilator but also it can totally abolish uterine contractile activity. These two properties suggest that this compound may be extremely im-

portant in regulating uterine blood flow and contractile activity during pregnancy.

We acknowledge the generous gift of prostacyclin (PGI<sub>2</sub>) from Dr. John Pike and The Upjohn Company, Kalamazoo, Michigan. In addition, we would like to thank the following individuals for the generous supply of vasoactive intestinal polypeptide: Dr. S. I. Said, University of Texas; Dr. V. Mutt, Karolinska Institute; and Dr. G. Kitze, National Institute of Arthritis, Metabolism, and Digestive Diseases.

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# "MENSTRUAL CRAMPS"

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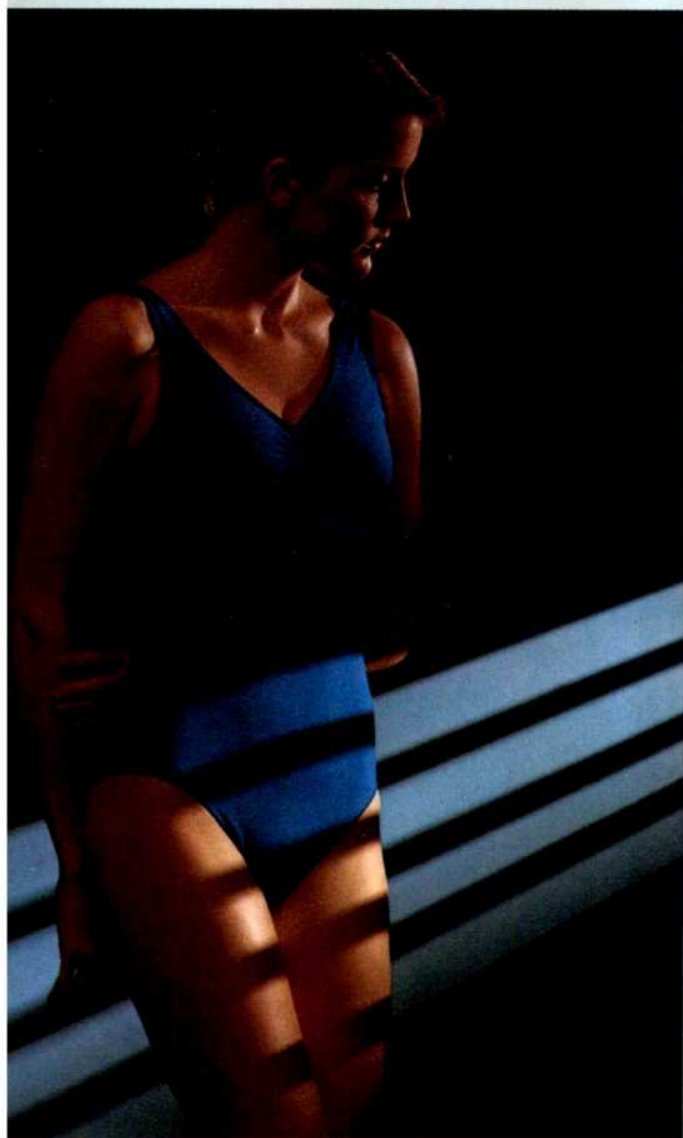


**A NEW  
THERAPEUTIC  
ALTERNATIVE\***

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# DYSMEN



275mg TABLETS

## NEW

# ANAPROX<sup>®</sup>

## (NAPROXEN SODIUM)

A new therapeutic alternative



# DYSMENORRHEA

## A NON-NARCOTIC, SINGLE-ENTITY ORAL ANALGESIC\*

New Anaprox® (naproxen sodium) is an effective, mid-range analgesic that has been proven to be useful in the treatment of primary dysmenorrhea. Anaprox represents a new therapeutic alternative for obstetricians/gynecologists who frequently encounter primary dysmenorrhea in practice.

## FULL THERAPEUTIC EFFECT IN ONE HOUR

With Anaprox, full therapeutic effect is reached within one hour. Peak plasma levels are attained in 1-2 hours. Anaprox also offers a longer half-life than other non-hormonal agents indicated for dysmenorrhea. This helps sustain relief of painful dysmenorrhea from dose to dose, whether administered t.i.d. or q.i.d.

## PROVIDES FAST, LONG-LASTING RELIEF OF PAIN AND UTERINE CRAMPING

Rapid absorption and fast onset of action help assure

rapid relief of the distressing symptoms of dysmenorrhea—pain and uterine cramping.

Anaprox  
Chemical Structure



## AN EFFECTIVE INHIBITOR OF PROSTAGLANDIN SYNTHETASE AND UTERINE CONTRACTILITY

Anaprox, a member of the arylacetic acid class of drugs, is an effective inhibitor of prostaglandin synthetase. In patients studied, it has been shown that Anaprox significantly reduces the level of prostaglandins in the uterus, which is accompanied by significant reduction in uterine contractions. Reduced con-

tractility results in decreased painful cramping of dysmenorrhea.

## WELL TOLERATED BY MOST PATIENTS

Anaprox was well tolerated in most patients studied. In fact, studies showed a lower incidence of GI and CNS side effects when it was compared with aspirin. With Anaprox, the most common adverse reactions were mild GI and CNS side effects, which occurred in approximately 10% and 11%, respectively. Use with caution in patients with impaired renal function. Because of adverse eye changes with NSAIs, periodic ophthalmic studies should be carried out. Peripheral edema has been observed in some patients. Because of its antipyretic properties, Anaprox may mask the signs and symptoms of infection. Before prescribing, please see warnings, precautions, and adverse reactions sections of full Prescribing Information.

Before initiating Anaprox therapy, it should be deter-

**\*Anaprox is a nonsteroidal anti-inflammatory agent, which has been developed as an analgesic and possesses antipyretic properties.**



# DYSMEN

mined that the patient is not pregnant. Anaprox should not be used concomitantly with the related drug Naprosyn® (naproxen), since both circulate in plasma as the naproxen anion and have the same side effects.

## EXTENSIVE CLINICAL DOCUMENTATION

Anaprox has been the subject of extensive clinical investigation in dysmenorrhea, having been studied in 212 patients over 496 menstrual cycles.

## EFFICACY DEMONSTRATED BY RELIEF OF MENSTRUAL PAIN

In eleven double-blind parallel or cross-over studies, and one single-blind study, involving 212 patients over 496 treatment courses, patients in 63% of the treatment courses derived "substantial to complete relief" and 11% experienced "mild to substantial relief" with Anaprox. Researchers concluded that "... a significant and beneficial effect of Anaprox on menstrual pain can be expected in about 75% of the treatment courses."

## EFFICACY DEMONSTRATED BY REDUCED NEED FOR ADDITIONAL ANALGESIA

The eleven double-blind comparison studies with placebo were conducted over 808 treatment courses. In 418 Anaprox treatment courses, only 16% required additional pain medication. In 390 placebo treatment courses, however, additional analgesia was required in 56% of the treatment courses.



## EFFICACY DEMONSTRATED BY LESS DAILY ACTIVITY INTERFERENCE

Continuation of patients' usual daily activities during painful menstruation was evaluated in a double-blind study involving 64 patients given Anaprox or placebo. Scores were totaled over three treatment courses. The study showed conclusively that those patients treated with Anaprox experienced substantially less interference with usual daily activities than those treated with placebo.

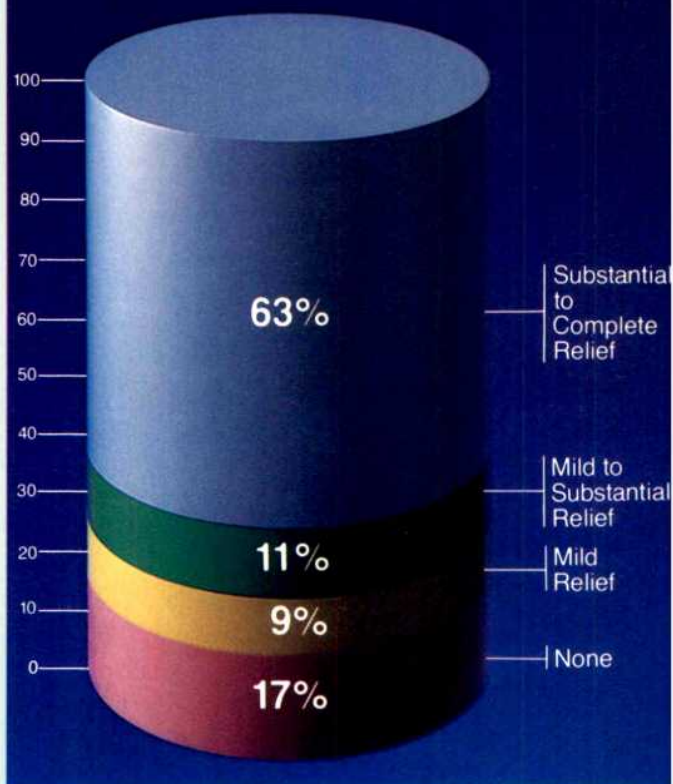
## EFFICACY DEMONSTRATED BY DECREASED INTRAUTERINE PRESSURE

Anaprox efficacy in dysmenorrhea has been further documented by measured decrease in uterine pressure. Measurement was conducted using a microballoon-tipped catheter introduced transcervically into the uterine cavity and connected to a recording system. Patients treated with Anaprox showed a significant decrease in resting pressure, amplitude, and frequency of uterine contractions.



# DYSPARRHEA

Degree of menstrual pain relief  
achieved with Anaprox



**NEW**

**ANAPROX<sup>®</sup>**  
**(NAPROXEN SODIUM)**

275mg TABLETS

**A new therapeutic alternative**

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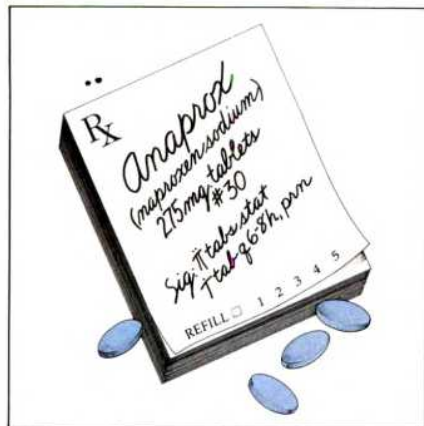
# FOR DYSMENORRHEA

## NEW

275 mg tablets

# ANAPROX®

(NAPROXEN SODIUM)



**Description:** ANAPROX filmcoated tablets for oral administration each contain 275 mg of naproxen sodium, which is equivalent to 250 mg naproxen with 25 mg (about 1 mEq) sodium. It is related to the arylacetic acid class of drugs. ANAPROX is a non-narcotic analgesic agent with anti-inflammatory and antipyretic actions. These properties have been demonstrated in classical animal test systems.

The chemical name of naproxen sodium is (-)-sodium 2-(6-methoxy-2-naphthyl) propionate.

Naproxen sodium is a white to creamy white, crystalline solid, freely soluble in water.

**Clinical Pharmacology:** Naproxen sodium is rapidly and completely absorbed from the gastrointestinal tract. After administration of naproxen sodium, peak plasma levels of naproxen anion are attained at 1-2 hours. The mean biological half-life of the anion in humans is approximately 13 hours, and at therapeutic levels it is greater than 99% albumin bound. Approximately 95% of the dose is excreted in the urine, primarily as naproxen, 6-0-desmethyl naproxen or their conjugates. The rate of excretion has been found to coincide closely with the rate of drug disappearance from the plasma. The drug does not induce metabolizing enzymes. Naproxen anion inhibits prostaglandin synthesis but beyond this its mode of action is unknown.

The drug was studied in patients with mild to moderate pain, and pain relief was obtained within 1 hour. It is not a narcotic and is not a CNS-acting drug. Controlled double-blind studies have demonstrated the analgesic properties of the drug in, for example, post-operative, post-partum, orthopedic and uterine contraction pain and dysmenorrhea. In dysmenorrheic patients, the drug reduces the level of prostaglandins in the uterus, leading to a reduction in the frequency and severity of uterine contractions. Analgesic action has been shown by such measures as reduction of pain intensity scores, increase in pain relief scores, decrease in numbers of patients requiring additional analgesic medication, and delay in time for required remediation. The analgesic effect has been found to last for up to 7 hours.

The drug was studied in patients with rheumatoid arthritis and osteoarthritis. It is not a corticosteroid. Improvement in patients treated for rheumatoid arthritis has been demonstrated by a reduction in joint swelling, a reduction in pain, a reduction in duration of morning stiffness, a reduction in disease activity as assessed by both the investigator and patient, and by increased mobility as demonstrated by a reduction in walking time.

In patients with osteoarthritis, the therapeutic action of the drug has been shown by a reduction in joint pain or tenderness, an increase in range of motion in knee joints, increased mobility as demonstrated by a reduction in walking time, and improvement in capacity to perform activities of daily living impaired by the disease.

In clinical studies in patients with rheumatoid arthritis and osteoarthritis, the drug has been shown to be comparable to aspirin in controlling the aforementioned measures of disease activity, but the frequency and severity of the milder gastrointestinal adverse effects (nausea, dyspepsia, heartburn) and nervous system adverse effects (tinnitus, dizziness, light-headedness) were less than in the aspirin-treated patients. It is not known whether the drug causes less peptic ulceration than aspirin.

The drug may be used safely in combination with gold salts and/or corticosteroids; however, in controlled clinical trials, when added to the regimen of patients receiving corticosteroids it did not appear to cause greater improvement over that seen with corticosteroids alone. Whether the drug could be used in conjunction with partially effective doses of corticosteroid for a "steroid-sparing" effect has not been adequately studied. When added to the regimen of patients receiving gold salts, the drug did result in greater improvement. Its use in combination with salicylates is not recommended because data are inadequate to demonstrate that the drug produces greater improvement over that achieved with aspirin alone. Further, there is some evidence that aspirin increases the rate of excretion of the drug.

Generally, improvement due to the drug has not been found to be dependent on age, sex, severity or duration of disease.

In <sup>51</sup>Cr blood loss and gastroscopy studies with normal volunteers, daily administration of 1100 mg of ANAPROX (naproxen sodium) has been demonstrated to cause statistically significantly less gastric bleeding and erosion than 3250 mg of aspirin.

**Indications and Usage:** ANAPROX® (naproxen sodium) is indicated in the relief of mild to moderate pain and for the treatment of primary dysmenorrhea.

It is also indicated for the treatment of the signs and symptoms of rheumatoid arthritis and osteoarthritis.

The safety and effectiveness of the drug in children have not been established.

**Contraindications:** Naproxen sodium is contraindicated in patients who have shown hypersensitivity to it. Because the potential exists for cross-sensitivity reactions, it should not be given to patients in whom aspirin or other non-steroidal, anti-inflammatory drugs induce the syndrome of asthma, rhinitis, or urticaria.

**Warnings:** Gastrointestinal bleeding, sometimes severe, and occasionally fatal, has been reported in patients receiving the drug. Among 960 patients treated for rheumatoid arthritis or osteoarthritis during the course of clinical trials in the United States (260 treated for more than two years), 16 cases of peptic ulceration were reported. More than half were on concomitant corticosteroid and/or salicylate therapy and about a third had a prior history of peptic ulcer. Gastrointestinal bleeding, including nine potentially serious cases, was also reported in this population. These were not always preceded by premonitory gastrointestinal symptoms. Although most of the patients with serious bleeding were receiving concomitant therapy and had a history of peptic ulcer disease, it should be kept in mind that the drug also has the potential for causing gastrointestinal bleeding on its own. Therefore, it should be administered to patients with active gastric and duodenal ulcers only under close supervision.

**Precautions: General:** ANAPROX® (NAPROXEN SODIUM) SHOULD NOT BE USED CONCOMITANTLY WITH THE RELATED DRUG NAPROSYN® (NAPROXEN) SINCE THEY BOTH CIRCULATE IN PLASMA AS THE NAPROXEN ANION.

Because of adverse eye findings in animal studies with drugs of this class, it is recommended that ophthalmic studies be carried out within a reasonable period of time after starting therapy and at periodic intervals thereafter if the drug is to be used for an extended period of time.

In chronic studies in laboratory animals, the drug has caused nephritis. Glomerular nephritis, interstitial nephritis and nephrotic syndrome have been reported. Since the drug is eliminated to a large extent from the body by urinary excretion via glomerular filtration, this drug should be used with great caution in patients with significantly impaired renal function and the monitoring of serum creatinine and/or creatinine clearance is advised in these patients. In clinical trials a few patients developed mild elevations in BUN accompanied by no other signs and symptoms.

If steroid dosage is reduced or eliminated during therapy, the steroid dosage should be reduced slowly and the patients must be observed closely for any evidence of adverse effects, including adrenal insufficiency and exacerbation of symptoms of arthritis.

Patients with initial hemoglobin values of 10 grams or less who are to receive long-term therapy should have hemoglobin values determined frequently.

Peripheral edema has been observed in some patients. Since each naproxen sodium tablet contains approximately 25 mg (about 1 mEq) of sodium, this should be considered in patients whose overall intake of sodium must be markedly restricted. It is possible that patients with questionable or compromised cardiac function may be at a greater risk when taking the drug.

**Information for Patients:** Caution should be exercised by patients whose activities require alertness if they experience drowsiness, dizziness, vertigo or depression during therapy with the drug.

**Drug Interactions:** *In vitro* studies have shown that naproxen anion, because of its affinity for protein, may displace from their binding sites other drugs which are also albumin-bound. Theoretically, the naproxen anion itself could likewise be displaced. Short-term controlled studies failed to show that taking the drug significantly affects prothrombin times when administered to individuals on coumarin-type anticoagulants. Caution is advised nonetheless, since interactions have been seen with other nonsteroidal agents of this class. Similarly, patients receiving the drug and a hydantoin, sulfonamide or sulfonylurea should be observed for signs of toxicity to these drugs.

Probenecid given concurrently increases naproxen anion plasma levels and extends its plasma half-life significantly. **Drug/Laboratory Test Interactions:** The drug may decrease platelet aggregation and prolong bleeding time. This effect should be kept in mind when bleeding times are determined.

The administration of the drug may result in increased urinary values for 17-ketogenic steroids because of an interaction between the drug and/or its metabolites with m-dinitro-benzene used in this assay. Although 17-hydroxy- corticosteroid measurements (Porter-Silber test) do not appear to be artifactually altered, it is suggested that therapy with the drug be temporarily discontinued 72 hours before adrenal function tests are performed.

The drug may interfere with some urinary assays of 5-hydroxy indoleacetic acid (5HIAA).

**Carcinogenesis:** A two-year study was performed in rats to evaluate the carcinogenic potential of the drug. No evidence of carcinogenicity was found.

**Pregnancy:** Teratogenic Effects: Pregnancy Category B. Reproduction studies have been performed in rats, rabbits

and mice at doses up to six times the human dose and have revealed no evidence of impaired fertility or harm to the fetus due to the drug. There are, however, no adequate and well-controlled studies in pregnant women. Because animal reproduction studies are not always predictive of human response, the drug should not be used during pregnancy unless clearly needed.

**Non-teratogenic Effects:** In rats, pregnancy was prolonged when the drug was given before the onset of labor; where it was given after the delivery process had begun, labor was protracted.

**Nursing Mothers:** Caution should be exercised if the drug is administered to a nursing woman since the naproxen anion has been found in the milk of lactating women at a concentration of approximately 1% of that found in the plasma.

**Pediatric Use:** Pediatric indications and dosage have not been established.

**Adverse Reactions:** Adverse reactions reported in controlled clinical trials in 960 patients treated for rheumatoid arthritis or osteoarthritis are listed below. In general, these reactions were reported 2 to 10 times more frequently than they were in studies in the 962 patients treated for mild to moderate pain or for dysmenorrhea.

**Incidence Greater Than 1%: Gastrointestinal:** The most frequent complaints reported related to the gastrointestinal tract. They were: constipation,\* heartburn,\* abdominal pain,\* nausea,\* dyspepsia, diarrhea, stomatitis.

**Central Nervous System:** Headache,\* dizziness,\* drowsiness,\* lightheadedness, vertigo.

**Dermatologic:** Itching (pruritis),\* skin eruptions,\* ecchymoses,\* sweating, purpura.

**Special Senses:** Tinnitus,\* hearing disturbances, visual disturbances.

**Cardiovascular:** Edema,\* dyspnea,\* palpitations.

**General:** Thirst.

\*Incidence of reported reaction between 3% and 9%. Those reactions occurring in less than 3% of the patients are unmarked.

**Incidence Less Than 1%: Probable Causal Relationship:** The following adverse reactions were reported less frequently than 1% during controlled clinical trials and through voluntary reports since marketing. The probability of a causal relationship exists between the drug and these adverse reactions: congestive heart failure, renal disease, glomerular nephritis, interstitial nephritis, nephrotic syndrome, abnormal liver function tests, hematuria, jaundice, thrombocytopenia, leukopenia, granulocytopenia, gastrointestinal bleeding, peptic ulceration with bleeding and/or perforation, hematemesis, melena, vomiting, eosinophilia, pyrexia (chills and fever), skin rashes, menstrual disorders, myalgia and muscle weakness, alopecia, inability to concentrate, depression, malaise, dream abnormalities.

**Causal Relationship Unknown:** Other reactions have been reported in circumstances in which a causal relationship could not be established. However, in these rarely reported events, the possibility cannot be excluded. Therefore these observations are being listed to serve as alerting information to the physicians: angioneurotic edema, agranulocytosis, aplastic anemia, hemolytic anemia, hypoglycemia, hyperglycemia, urticaria.

**Overdosage:** Significant overdosage may be characterized by drowsiness, heartburn, indigestion, nausea or vomiting. Because naproxen sodium may be rapidly absorbed, high and early blood levels should be anticipated. No evidence of toxicity or late sequelae have been reported 5 to 15 months after ingestion for three to seven days of doses equivalent to up to 3,300 mg of naproxen sodium. One patient ingested a single dose equivalent to 27.5 g of naproxen sodium and experienced mild nausea and indigestion. It is not known what dose of the drug would be life threatening. The oral LD<sub>50</sub> of the drug is 543 mg/kg in rats, 1234 mg/kg in mice, 4110 mg/kg in hamsters and greater than 1000 mg/kg in dogs.

Should a patient ingest a large number of tablets, accidentally or purposefully, the stomach may be emptied and usual supportive measures employed. Animal studies suggest that the prompt administration of 5 grams of activated charcoal would tend to reduce markedly the absorption of the drug. It is not known if the drug is dialyzable.

**Dosage and Administration: For Mild to Moderate Pain and Dysmenorrhea:** The recommended starting dose is two 275 mg tablets, followed by one 275 mg tablet every 6 to 8 hours, as required. The total daily dose should not exceed 5 tablets (1,375 mg).

**For Rheumatoid Arthritis and Osteoarthritis:** The recommended starting dose in adults is one 275 mg tablet twice daily (morning and evening) or one 275 mg tablet in the morning and two 275 mg tablets in the evening. During long-term administration, the dose may be adjusted up or down depending on the clinical response of the patient. A lower daily dose may suffice for long-term administration. Daily doses higher than 1100 mg in these indications have not been studied. The morning and evening doses do not have to be equal in size and the administration of the drug more frequently than twice daily is not necessary. Symptomatic improvement in arthritis usually begins within two weeks. However, if improvement is not seen within this period, a trial for an additional two weeks should be considered.

**How Supplied:** ANAPROX® (naproxen sodium) is available in filmcoated tablets of 275 mg (light blue), in bottles of 100 tablets (NDC 18393-274-42) and 500 tablets (NDC 18393-274-62). Store at room temperature in well-closed containers.



Syntex Puerto Rico, Inc.  
Humacao, P.R. 00661



## FETUS, PLACENTA, AND NEWBORN

### Effects of maternal acetonuria and low pregnancy weight gain on children's psychomotor development

RICHARD L. NAEYE, M.D.

RONALD A. CHEZ, M.D.

*Hershey, Pennsylvania*

Data were analyzed from a large prospective study to determine if children had an excess of psychomotor or IQ impairment when their mothers had either acetonuria, weight loss, or low weight gain during pregnancy. The effects of these maternal factors on children's development were assessed by a variety of mental and motor tests from the newborn period to 7 years of age. No consistent impairments were found at any age. (AM. J. OBSTET. GYNECOL. 139:189, 1981.)

CHURCHILL AND ASSOCIATES<sup>1, 2</sup> reported that children whose diabetic and nondiabetic mothers had acetonuria during pregnancy had lower mental and motor scores at 8 months and lower IQ values at 4 years of age than children not so exposed. Stebbens and colleagues<sup>3</sup> examined offspring of diabetic women and found a significant association of gestational acetonuria with lower IQ values at 5 years of age. A more recent study did not confirm Stebbens's findings.<sup>4</sup> The present study continued the latter investigation by examining the relationship of maternal acetonuria, weight loss, and low weight gain during pregnancy to the psychomotor performances and IQ values of neonates and older children of nondiabetic mothers. Unlike the earlier studies the present study controlled for a number

of nonnutritional factors that affect psychomotor performance.

#### Patients and methods

The Collaborative Perinatal Project of the National Institute of Neurological and Communicative Disorders and Stroke provides an opportunity to study pregnancy and its long-term outcome. It prospectively followed the course of 53,518 pregnancies in the United States, between 1959 and 1966, and recorded events of gestation, labor, delivery, and the neonatal period as well as children's development to 8 years of age.<sup>5, 6</sup> With the data from the study we analyzed the effects of maternal acetonuria, weight loss, and low weight gain during pregnancy on children's psychomotor development. The methods used to evaluate psychomotor development and IQ are described in the published manual for the Collaborative Study.<sup>6</sup>

All singleton infants born alive after 20 or more weeks of gestation were entered in the study. To reduce the number of variables to be considered we excluded from the study cases with the following non-nutritional factors that can affect psychomotor development: major congenital malformations, Down's syndrome, inborn errors of metabolism, head trauma during or after birth, and lead intoxication in the child.

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**Table I.** Psychomotor functions in white children born at term

| Psychomotor function       | Acetonuria         |                  | Maternal weight during pregnancy |                  |
|----------------------------|--------------------|------------------|----------------------------------|------------------|
|                            | Absent (%)         | Present (%)      | Gain of 22 to 32 pounds (%)      | Weight loss (%)  |
| <i>Neonatal period:</i>    |                    |                  |                                  |                  |
| Meconium in amniotic fluid | 19.2 (668)         | 19.5 (216)       | 18.5 (366)                       | 24.0 (12)        |
| 1-minute Apgar score 0-3   | 3.7 (129)          | 3.3 (36)         | 3.8 (70)                         | 8.5 (4)          |
| 5-minute Apgar score 0-3   | 0.6 (21)           | 0.8 (9)          | 0.7 (14)                         | 0                |
| Abnormal Moro reflex       | 3.7 (127)          | 4.3 (48)         | 3.7 (72)                         | 12.0 (6)*        |
| Other abnormal reflexes    | 3.5 (121)          | 5.0 (55)         | 3.9 (76)                         | 4.0 (2)          |
| Hypotonia                  | 3.6 (123)          | 6.2 (69)†        | 5.1 (100)                        | 10.0 (5)         |
| Hypertonia                 | 1.9 (65)           | 2.2 (24)         | 1.2 (24)                         | 4.0 (2)          |
| Myoclonus                  | 0.4 (14)           | 0.2 (2)          | 0.5 (9)                          | 2.0 (1)          |
| Abnormal suck              | 1.3 (45)           | 1.3 (14)         | 1.4 (28)                         | 2.0 (1)          |
| Abnormal cry               | 2.2 (75)           | 2.5 (28)         | 2.0 (39)                         | 6.0 (3)          |
| Jitters                    | 4.8 (168)          | 5.5 (61)         | 4.9 (96)                         | 8.0 (4)          |
| Nystagmus                  | 0.1 (5)            | 0.4 (4)          | 0.2 (4)                          | 2.0 (1)          |
| Seizures                   | 0.2 (7)            | 0.5 (5)          | 0.2 (4)                          | 0                |
| Transient abnormality      | 7.4 (257)          | 8.2 (91)         | 7.7 (151)                        | 20.0 (10)‡       |
| <i>Age 8 months:</i>       |                    |                  |                                  |                  |
| Mean Bayley motor score    | 34.2 ± 4.5 (1,819) | 34.2 ± 4.0 (576) | 33.9 ± 4.4 (6,133)               | 33.2 ± 5.1 (123) |
| Mean Bayley mental score   | 80.2 ± 4.9 (1,817) | 80.3 ± 3.7 (577) | 80.3 ± 4.7 (6,114)               | 80.1 ± 4.2 (169) |

Numbers of cases are in parentheses. Acetonuria was recorded as present when it was 1+ or greater. All P values except those specified were >0.1.

\*P < 0.05.

†P < 0.001.

‡P < 0.01.

Cases were also excluded from the analyses if either parent was mentally retarded or the mother had any of the following: hypothyroidism, a convulsive disorder, diabetes mellitus, or alcoholism. Data in the study were stratified by race, gestational age at birth, and a socioeconomic index which combined scores for education, occupation, and family income.<sup>7</sup> A value below 4 was considered to be low. Gestational age was calculated at birth from the start of the last menstrual period. Race was established by mother's self-identification. Acetonuria was recorded when the reading was 1+ or greater on one or more urine specimens examined during antenatal clinic visits and at admission for delivery. The chi-square test, Student's t test, and the three-way F test with one-way analysis of variance were the statistical methods used in the study.

### Results

In white women neither gestational acetonuria, weight loss, nor low weight gain were associated with any consistent evidence of mental or motor impairments in offspring during the first year of life (Tables I and II). When compared to children whose mothers had weight gains of 22 to 32 pounds, two of the seven tests at 4 years of age were failed more often by children whose mothers lost weight during pregnancy (Table II).

- Combining acetonuria with low weight gain or

weight loss produced no consistent evidence of mental or motor impairments at any age. As independent variables, neither acetonuria, low weight gain, nor pregnancy weight loss were associated with abnormal IQ values at 4 years of age (Tables III and IV).

Although not shown in the tables, acetonuria, low maternal pregnancy weight gain, and pregnancy weight loss had no significant effect on IQ values and other measures of the psychomotor performance of black children.

### Comment

The reported association of maternal acetonuria during pregnancy with subsequent reduction of children's IQ values has influenced the clinical care of pregnant women. The avoidance of acetonuria has been one reason advanced to maintain appropriate weight gains during pregnancy, avoid weight-reducing diets, and maintain a substantial food intake during the nausea and vomiting phase of early gestation.

We found that maternal acetonuria, low weight gain, and weight loss during pregnancy had little or no effect on neonatal, infant, and childhood psychomotor functions including IQ. These data were extracted from the same population used in the original study that claimed lowered IQ values following acetonuria.<sup>1,2</sup> Our results differ from those in the original study because we controlled for a number of nonnutritional factors that



Table II. Psychomotor functions in term white children

| Age                               | Gestational acetonuria |            | Maternal pregnancy weight change |             |
|-----------------------------------|------------------------|------------|----------------------------------|-------------|
|                                   | Absent                 | Present    | Gain of 22 to 32 pounds          | Weight loss |
| <i>One year:</i>                  |                        |            |                                  |             |
| Hypotonia                         | 4.6 (94)               | 3.3 (21)   | 4.2 (284)                        | 4.3 (97)    |
| Cranial nerve abnormality, facial | 0.7 (14)               | 1.3 (8)    | 1.1 (77)                         | 1.6 (3)     |
| Paraplegia                        | 1.2 (25)               | 1.6 (10)   | 1.2 (80)                         | 2.1 (4)     |
| Tetraplegia                       | 1.2 (25)               | 0.8 (5)    | 0.7 (50)                         | 1.6 (3)     |
| Ataxia                            | 0.3 (5)                | 0.2 (1)    | 0.2 (13)                         | 0.5 (1)     |
| Nystagmus                         | 0.1 (2)                | 1.1 (7)*   | 0.2 (16)                         | 0.5 (1)     |
| Spastic paresis                   | 0.3 (6)                | 0.5 (3)    | 0.7 (45)                         | 0.5 (1)     |
| Esotropia                         | 4.4 (80)               | 3.6 (23)   | 4.3 (295)                        | 3.2 (6)     |
| Exotropia                         | 0.2 (4)                | 0.2 (1)    | 1.8 (120)                        | 1.6 (3)     |
| Strabismus                        |                        |            |                                  |             |
| Alternating internal              | 1.5 (30)               | 1.9 (121)  | 1.6 (107)                        | 1.6 (3)     |
| Alternating external              | 0.2 (3)                | 0.8 (5)†   | 0.1 (9)                          | 0           |
| <i>Four years:</i>                |                        |            |                                  |             |
| Failed line walk                  | 6.7 (109)              | 6.6 (35)   | 5.2 (261)                        | 4.4 (6)     |
| Failed ball catch                 | 43.4 (712)             | 40.0 (215) | 38.2 (1,964)                     | 32.2 (46)   |
| Failed Wallin pegboard            |                        |            |                                  |             |
| Right hand                        | 1.3 (21)               | 1.7 (9)    | 1.3 (67)                         | 0           |
| Left hand                         | 2.7 (44)               | 2.6 (14)   | 2.2 (113)                        | 3.5 (5)     |
| Failed copy circle                | 3.1 (52)               | 3.7 (20)   | 2.5 (128)                        | 8.4 (12)‡   |
| Failed Proteus maze               | 25.7 (422)             | 27.0 (146) | 26.6 (1,365)                     | 37.9 (53)†  |
| Failed string beads               | 4.2 (69)               | 4.4 (24)   | 4.0 (208)                        | 6.3 (9)     |

Numbers of cases are in parentheses. All P values except those specified were >0.1.

\*P < 0.01.

†P < 0.05.

‡P < 0.001.

Table III. Mothers' pregnancy weight gains and their children's IQ values at 4 years of age

|                                   | Mean IQ values at 4 years of age |                     |                      |                      |
|-----------------------------------|----------------------------------|---------------------|----------------------|----------------------|
|                                   | Mother's pregnancy weight change |                     |                      |                      |
|                                   | Weight loss                      | Gain of 1-10 pounds | Gain of 11-21 pounds | Gain of 22-32 pounds |
| <i>Socioeconomic Index 0-3.9:</i> |                                  |                     |                      |                      |
| <38 weeks' gestation              | 103 ± 7 (2)                      | 101 ± 15 (5)        | 96 ± 19 (11)         | 96 ± 11 (17)         |
| 38-44 weeks' gestation            | 93 ± 17 (13)                     | 92 ± 14 (35)        | 96 ± 14 (129)        | 95 ± 15 (201)        |
| <i>Socioeconomic Index 4-5.9:</i> |                                  |                     |                      |                      |
| <38 weeks' gestation              | 96 ± 8 (3)                       | 92 ± 6 (3)          | 97 ± 15 (16)         | 100 ± 15 (12)        |
| 38-44 weeks' gestation            | 101 ± 10 (5)                     | 98 ± 20 (30)        | 100 ± 15 (151)       | 100 ± 15 (222)       |
| <i>Socioeconomic Index 6-7.9:</i> |                                  |                     |                      |                      |
| <38 weeks' gestation              | 92 ± 13 (4)                      | 106 ± 7 (5)         | 106 ± 16 (22)        | 99 ± 17 (16)         |
| 38-44 weeks' gestation            | 104 ± 9 (3)                      | 103 ± 12 (21)       | 107 ± 15 (190)       | 106 ± 16 (295)       |
| <i>Socioeconomic Index 8-9.5:</i> |                                  |                     |                      |                      |
| <38 weeks' gestation              | 118 ± 9 (2)                      | 123 ± 7 (3)         | 106 ± 15 (15)        | 111 ± 10 (19)        |
| 38-44 weeks' gestation            | 115 ± 8 (2)                      | 110 ± 14 (17)       | 115 ± 16 (291)       | 115 ± 10 (423)       |

Figures in parentheses denote number of cases. All P values comparing IQ values in the categories <22 pounds were >0.1 compared with IQ values in the category 22 to 32 pounds.

influence psychomotor development and IQ.

Ketone bodies are probably normally presented to the fetal brain at various times during most pregnancies. After an overnight fast, maternal ketone body concentrations are about threefold greater in pregnant than in nonpregnant women; ketonuria will often occur in the pregnant woman.<sup>8, 9</sup> In a recent study,

Coëtze and associates<sup>9</sup> found that urine concentrations of ketone bodies may abruptly increase in the presence of less than a twofold increase in blood concentrations. Further, this change in blood levels is at the upper limit of normal range. Therefore, acetonuria in normal pregnancy probably does not usually signify that any abnormality is present.

Table IV. Mothers' gestational acetonuria and their children's IQ values at 4 years of age

|                                   | Mean IQ values         |                |                |
|-----------------------------------|------------------------|----------------|----------------|
|                                   | Trimester of pregnancy |                |                |
|                                   | First                  | Second         | Third          |
| <i>Socioeconomic Index 0-3.9:</i> |                        |                |                |
| <38 weeks' gestation              |                        |                |                |
| No acetonuria                     | 96 ± 12 (12)           | 91 ± 13 (21)   | 89 ± 13 (26)   |
| 2+ or more acetonuria             | —                      | 85 ± 11 (4)    | 92 ± 10 (7)    |
| 38-44 weeks' gestation            |                        |                |                |
| No acetonuria                     | 97 ± 11 (69)           | 95 ± 14 (239)  | 94 ± 14 (241)  |
| 2+ or more acetonuria             | 103 ± 13 (7)           | 102 ± 10 (14)  | 92 ± 13 (23)   |
| <i>Socioeconomic Index 4-5.9:</i> |                        |                |                |
| <38 weeks' gestation              |                        |                |                |
| No acetonuria                     | 98 ± 12 (7)            | 96 ± 15 (33)   | 94 ± 12 (29)   |
| 2+ or more acetonuria             | 101 ± 10 (2)           | 100 ± 8 (2)    | 102 ± 11 (7)   |
| 38-44 weeks' gestation            |                        |                |                |
| No acetonuria                     | 103 ± 12 (125)         | 98 ± 15 (282)  | 99 ± 14 (269)  |
| 2+ or more acetonuria             | 103 ± 13 (15)          | 93 ± 14 (16)   | 100 ± 12 (31)  |
| <i>Socioeconomic Index 6-9.5:</i> |                        |                |                |
| <38 weeks' gestation              |                        |                |                |
| No acetonuria                     | 108 ± 19 (59)          | 111 ± 17 (61)  | 106 ± 19 (65)  |
| 2+ or more acetonuria             | 120 ± 16 (8)           | 113 ± 15 (10)  | 114 ± 10 (18)  |
| 38-44 weeks' gestation            |                        |                |                |
| No acetonuria                     | 111 ± 15 (755)         | 110 ± 16 (878) | 110 ± 15 (617) |
| 2+ or more acetonuria             | 109 ± 13 (25)          | 101 ± 28 (22)  | 110 ± 18 (139) |

Numbers of cases are in parentheses. All of the P values comparing IQ values in the acetonuria category were greater than 0.1 compared with the no acetonuria category.

Mothers have an increased dependence on fat stores for fuel during the second half of pregnancy.<sup>10</sup> Associated increases in lipase activity and in levels of circulating free fatty acids and glycerol enhance the production of acetoacetyl coenzyme A and, therefore, ketogenesis.<sup>9, 10</sup> Ketones are transferred across the placenta and are probably a normal fuel for the human fetal brain, particularly during late gestation.<sup>11</sup> Levels of the enzymes required to metabolize ketone bodies increase in fetal brains during the second half of pregnancy.<sup>12</sup> In vitro studies on the human brain indicate that 3-hydroxybutyrate can be oxidized.<sup>13</sup> Neonatal brains use ketone bodies as fuel.<sup>14</sup>

Findings in the current study received indirect support from the studies of Stein and colleagues<sup>15</sup> of the Dutch famine of 1944 and 1945. They found no significant psychomotor impairments in military recruits who had been exposed during different periods of gestation to severe maternal food restrictions. Data on maternal acetonuria were not available, but hyperketonemia was probably more frequent in the famine victims than in the nonfamine control subjects.

Ms. Ellen C. Peters performed the statistical analyses in the study. Informed consent was obtained for the data collection.

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# Antepartum fetal heart rate testing

## VII. The significance of fetal bradycardia

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Antepartum fetal heart rate testing (AFHRT) was utilized in the assessment of fetal status on a large clinical service. The nonstress test (NST) was used as the primary approach. The contraction stress test (CST) was used in the face of persistent nonreactivity. The significance of fetal bradycardia occurring during AFHRT was assessed. Bradycardia was defined as a fetal heart rate (FHR) of 90 bpm or a reduction in FHR of 40 bpm below baseline, for 60 seconds or greater. In a 2-year period, there were 28 cases of bradycardia during 3,754 tests. A total of 24 cases were sent for delivery on the same day while four cases were managed in variable fashion. In 16 cases the tests were classified as nonreactive (abnormal) and the patients were sent for delivery on the same day. There was a 50% incidence of emergency delivery for fetal distress in labor in this group. In eight cases the tests were classified as reactive (normal) and the patients were sent for delivery on the same day. The incidence of fetal distress in labor with emergency delivery was 50%. Five of 10 patients with decreased amniotic fluid volume either on ultrasonic evaluation or by clinical estimation developed fetal distress in labor. There were three cases of congenital anomalies and one case of intrauterine growth retardation which exhibited diminished amniotic fluid. The occurrence of fetal bradycardia during AFHRT denotes a fetus at increased risk of developing fetal distress in labor. This type of test should be considered abnormal and consideration should be given to delivery of the fetus. (AM. J. OBSTET. GYNECOL. 139:194, 1981.)

THE USE OF antepartum fetal heart rate testing (AFHRT) to assess the status of the fetus at risk is widespread in current obstetric practice. Although the contraction stress test (CST) is still being used, the nonstress test (NST) has supplanted the CST as the primary method of fetal assessment in many institutions. At the Los Angeles County/University of Southern California Medical Center, Women's Hospital, the NST has been the primary approach for approximately 5 years.<sup>1-3</sup>

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Table I. Primary indications for test

| Indication                   | No. of patients |
|------------------------------|-----------------|
| Suspected postdates          | 19              |
| Suspected growth retardation | 4               |
| Diabetes mellitus            | 3               |
| Decreased fetal movement     | 1               |
| Von Gierke's disease         | 1               |
| Total                        | 28              |

Since bradycardia alone or in combination with excessive uterine activity (hyperstimulation) has been reported in 5% to 10% of tests,<sup>4</sup> we sought to evaluate the incidence and possible significance of fetal bradycardia. Oligohydramnios also has been implicated as a possible contributing factor and the relationship of amniotic fluid volume in these patients is presented.

### Material and methods

All AFHRT recordings exhibiting any decelerations were reviewed for a period of 2 years, July, 1977,

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Table II. Reactive NST

| Case No. | Diagnosis | Amniotic fluid |          | Outcome  |            |                                                                    |
|----------|-----------|----------------|----------|----------|------------|--------------------------------------------------------------------|
|          |           | Volume         | Meconium | Delivery | Indication | FHR pattern                                                        |
| 1        | IUGR      | ↓              | 0        | C/S      | FD*        | Bradycardia                                                        |
| 2        | IUGR      | ↓              | 0        | C/S      | FD†        | Bradycardia, severe variable decelerations                         |
| 3        | PD        | ↓              | 0        | C/S      | FTP‡       | Normal                                                             |
| 4        | PD        | ↓              | NR       | C/S      | FTP        | Intermittent late decelerations                                    |
| 5        | PD        | ↓              | +        | C/S      | FD         | Bradycardia, late decelerations, and severe variable decelerations |
| 6        | PD        | NR             | NR       | C/S      | FD         | Prolonged bradycardia                                              |
| 7        | PD        | N              | 0        | NSVD     |            | Normal                                                             |
| 8        | PD        | N              | 0        | C/S      | FTP        | Normal                                                             |

IUGR: Suspected intrauterine growth retardation; PD: suspected postdates; C/S: cesarean section; N: normal; ↓: decreased; FD: fetal distress; FTP: failure to progress in labor; NSVD: normal spontaneous vaginal delivery.

\*Congenital rubella syndrome.

†Severe IUGR, cord around thorax.

‡Down's syndrome.

Table III. Nonreactive NST

| Case No. | Diagnosis    | CST  | Amniotic fluid |          | Outcome  |                          |                                 |
|----------|--------------|------|----------------|----------|----------|--------------------------|---------------------------------|
|          |              |      | Volume         | Meconium | Delivery | Indication               | FHR pattern                     |
| 1        | DM           | Δ    | N              | 0        | C/S      | FD                       | Late decelerations              |
| 2        | DM           | +    | NR             | NR       | C/S      | Face presentation<br>FTP | Variable decelerations          |
| 3        | IUGR         | Δ    | ↓              | 0        | NSVD     |                          | N                               |
| 4        | IUGR         | Neg. | ↓              | +        | C/S      | Elective*                |                                 |
| 5        | von Gierke's | Δ    | N              | 0        | NSVD     |                          | N                               |
| 6        | ↓FM          | Δ    | NR             | NR       | C/S      | FD                       | Prolonged bradycardia           |
| 7        | PD           | Neg. | ↓              | +        | C/S      | FD                       | Loss of variability, falling pH |
| 8        | PD           | Δ    | N              | 0        | C/S      | FD                       | Prolonged bradycardia           |
| 9        | PD           | Δ    | N              | 0        | NSVD     | †                        | N                               |
| 10       | PD           | Δ    | NR             | NR       | C/S      | FD†                      | Severe variable decelerations   |
| 11       | PD           | Δ    | ↓              | 0        | C/S      | FD                       | Prolonged bradycardia           |
| 12       | PD           | Δ    | N              | 0        | Vacuum   | FD                       | Prolonged bradycardia           |
| 13       | PD           | Δ    | ↓              | 0        | C/S      | Repeat†                  |                                 |
| 14       | PD           | Δ    | ↓              | +        | NSVD     |                          | N                               |
| 15       | PD           | Δ    | ↓              | +        | C/S      | FTP                      | N                               |
| 16       | PD           | Neg. | ↓              | +        | C/S      | FD                       | Late decelerations, low pH      |

DM: Diabetes mellitus; Δ: CST not done; NR: not recorded. For other abbreviations see Table II footnote.

\*Spina bifida, meningomyelocele.

†Nuchal cord.

through June, 1979. The indications for testing and the procedure, primarily the NST, have been previously reported.<sup>3</sup> A "reactive" test is defined as the occurrence of two accelerations of 15 bpm above the baseline FHR level, lasting for 15 seconds, associated with fetal movement, in a 20-minute period. A "non-reactive" test is one that does not meet the aforementioned criteria. At the conclusion of all tests, fundal and suprapubic pressure was manually applied for 1 minute. This was done in an attempt to elicit a deceleration, which might possibly indicate cord entrapment.<sup>3</sup>

In this evaluation bradycardia was defined as an absolute heart rate below 90 bpm for 1 minute or longer or a reduction in heart rate of greater than 40 bpm

below the baseline level for 1 minute or longer. Twenty-eight patients in a total of 1,725 (1.6%) met the criteria for bradycardia as defined above. The total number of tests performed in this time period was 3,754, for an incidence of bradycardia of 7/1,000 tests. Twenty-four of the patients whose fetuses exhibited bradycardia were sent for delivery on the same day since it was felt that bradycardia of this degree was potentially ominous. The remaining four cases were managed in variable ways which will be discussed individually. A trial of labor was attempted in all but two cases, the first being a patient with a previous cesarean section and the other a case in which the antepartum diagnosis of spina bifida and meningomyelocele had

been made. Twenty-five patients were electronically monitored during labor. The primary indication for testing is illustrated in Table I.

Amniotic fluid volume was assessed: (1) by ultrasonic scanning, (2) at the time of amniotomy, or (3) at cesarean section. Amniotic fluid volume was considered normal if, at the time of ultrasonic examination, a pocket of fluid of at least 1 cm in depth was seen. The assessment of fluid volume at the time of amniotomy or delivery was noted as decreased or normal by the resident physician. The presence or absence of meconium staining of amniotic fluid was noted.

The clinical diagnosis of fetal distress in labor was defined as abnormal FHR patterns<sup>5, 6</sup> and/or fetal acidosis determined by scalp blood sampling which led to emergency delivery. Abnormal patterns included late decelerations, severe variable decelerations, prolonged bradycardia, tachycardia, and loss of baseline beat-to-beat variability. Periodic FHR patterns were defined according to the method of Kubli and associates.<sup>5</sup> Fetal distress according to pH determinations was defined as a progressively falling trend or when the absolute pH was below 7.20.

## Results

The outcome of the 24 patients delivered on the same day as the test was performed will be discussed at this point. The four patients who were not delivered the same day will be discussed individually.

**Mode of delivery.** Of the 24 cases under consideration, 11 patients underwent cesarean section and one had a vacuum extraction delivery for fetal distress in labor. Thus, 50% of cases required emergency delivery for fetal distress. Seven additional patients required cesarean section for various indications (Tables II and III) and only five patients experienced a spontaneous vaginal delivery.

**Reactive tests (Table II).** There were eight tests that were classified as "reactive." Half of the cases in this group underwent cesarean section for fetal distress in labor. These four cases all exhibited prolonged bradycardia or severe variable deceleration patterns during labor.

Three cesarean sections were performed for the diagnosis of failure to progress in labor, and there was only one normal spontaneous vaginal delivery. There were two neonates with a congenital anomaly in this group. One manifested severe congenital rubella syndrome (Case No. 1) and the other had Down's syndrome (trisomy 21) (Case No. 3).

**Nonreactive tests (Table III).** There were 16 tests classified as "nonreactive," two thirds of all cases of bradycardia. Half of these cases (eight of 16) under-

went emergency delivery as a result of the diagnosis of fetal distress in labor. Seven were delivered by cesarean section and one, by vacuum extraction. Five of these eight cases demonstrated moderate to severe variable decelerations and/or prolonged bradycardia during labor. The remaining three cases exhibited late decelerations, loss of beat-to-beat variability, and progressive fetal acidosis. In two of the three cases with such patterns, a negative CST had followed the nonreactive NST (Cases Nos. 7 and 16, Table III).

Four other cesarean sections were performed in the nonreactive pattern group: one elective repeat cesarean section (Case No. 13), one elective cesarean section in a fetus with what was thought to be a potentially repairable meningocele (Case No. 4), one for face presentation with lack of progress of labor (Case No. 2), and one with failure of labor to progress (Case No. 15). In Case No. 2, with a face presentation, a positive CST followed a nonreactive NST and there were no late decelerations during labor. Mild variable decelerations did occur, but an infant with normal Apgar scores was delivered. In this group of 14 patients in whom a trial of labor occurred, only four patients were delivered vaginally.

**Amniotic fluid.** There were 20 cases in which the amount of amniotic fluid and the presence or absence of meconium was noted.

Twelve patients (60%) were judged to have a diminished amount of amniotic fluid and two of these patients underwent elective cesarean delivery. In the 10 patients undergoing labor, five were delivered by cesarean section for fetal distress. Of the eight patients judged to have normal amniotic fluid volume, fetal distress was demonstrated in four during labor.

Decreased amniotic fluid volume was noted in the three cases in which the fetus had a congenital anomaly and in a fourth case in which there was severe intrauterine growth retardation associated with maternal hypertension.

The presence or absence of meconium in amniotic fluid could not be related to the occurrence of fetal distress in labor (Tables II and III).

**Umbilical cord.** The position of the umbilical cord at delivery was recorded in all cases. There were three cases with a nuchal umbilical cord. In one of these cases (No. 10, Table III), the cord was wrapped tightly around the neck of the fetus four times and cesarean section was performed because of repetitive severe variable decelerations. In the remaining two cases one loop of cord was around the neck of the fetus. One patient was delivered by elective repeat cesarean section and there was one normal vaginal delivery. In one case (No. 2, Table II) the umbilical cord was wrapped



tightly around the thorax of the fetus at delivery. This infant was severely growth retarded and had decreased amniotic fluid volume. Cesarean section was performed because of severe variable decelerations and repeated prolonged bradycardia in this case.

**Fundal and suprapubic pressure.** In four of the 24 cases in the study group, the bradycardia was elicited in association with fundal and suprapubic pressure applied at the conclusion of testing. All four of these cases were judged to have decreased amniotic fluid (Table II, No. 3, and Table III, Nos. 7, 11, and 16). Three of these four underwent cesarean section for fetal distress and each of these three had nonreactive patterns, two of which were followed by a negative CST.

**Results in cases not delivered on the same day as AFHRT.** There were four cases which were not managed on the basis of intervention and delivery on the same day. Their management and results will be discussed individually.

*Case No. 1.* C. B. was referred for testing at 38 weeks' gestation, by inexact estimation of dates, because of Class A diabetes mellitus. The NST pattern was reactive, with one episode of bradycardia occurring during the test. No deceleration was noted following fundal and suprapubic pressure. The cervix was unfavorable for induction of labor and, because of the uncertain gestational age, an amniocentesis was performed to assess lung maturity. On ultrasonic scan, there was adequate amniotic fluid, and no gross fetal abnormalities were noted; the amniotic fluid was clear at amniocentesis. Repeat AFHRT 24 hours later was reactive, and no decelerations were noted. Serum unconjugated estriol levels were rising. The lecithin/sphingomyelin ratio was 2:1 but in view of an unfavorable cervix induction of labor was not undertaken. The patient underwent induction of labor 6 days following AFHRT, at which time the FHR in labor exhibited repetitive episodes of prolonged bradycardia. The patient was delivered by cesarean section for fetal distress.

*Case No. 2.* L. C. was referred for AFHRT because of suspected intrauterine growth retardation. The test was classified as reactive with one episode of bradycardia. Amniotic fluid volume was not recorded at this point and a repeat test 24 hours later was reactive without any decelerations. On the day following the last test, the patient was admitted in spontaneous labor. The FHR remained normal during labor. A growth-retarded infant was delivered vaginally without complications. No record of amniotic fluid or meconium was noted on the chart.

*Case No. 3.* R. R. was referred for AFHRT because of suspected postterm pregnancy. The AFHRT was

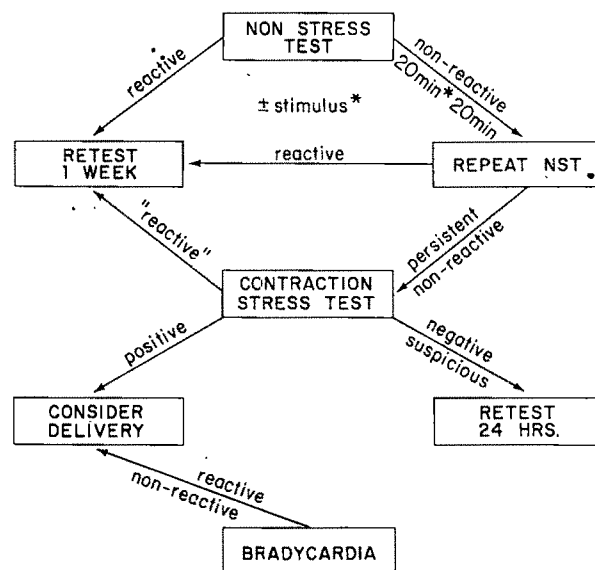


Fig. 1. AFHRT flow sheet at Cornell University Medical Center, 1980.

classified as reactive with one episode of bradycardia. Ultrasonic examination revealed decreased amniotic fluid. A repeat test within 24 hours was again interpreted as reactive, but no deceleration was noted. This patient was admitted in active labor the following day and was rapidly delivered vaginally of a depressed infant with Apgar scores of 1 and 1, respectively, at 1 and 5 minutes. A diagnosis of abruptio placentae was made following delivery. Since delivery was imminent on admission intrapartum monitoring was not instituted.

*Case No. 4.* M. M. was referred for AFHRT because of suspected postterm pregnancy. The AFHRT was interpreted as nonreactive and was followed by a negative CST and a prolonged bradycardia. A repeat test in 24 hours again was nonreactive with a negative CST but no bradycardia. This patient was admitted in advanced labor 36 hours following the last antepartum test. Intrapartum fetal monitoring was not used and an infant with Apgar scores of 4 at 1 minute and 9 at 5 minutes was delivered vaginally.

The occurrence of prolonged bradycardia during AFHRT seemingly identifies a fetus at high risk for developing distress in labor. The 50% incidence of operative delivery for fetal distress in labor was identical for tests classified as reactive and nonreactive. The three cases exhibiting late deceleration patterns or fetal acidosis in labor all had preceding nonreactive NSTs. Fetal distress in labor occurred in two of three cases with a negative CST following a nonreactive NST.

Decreased amniotic fluid was noted in 10 cases, and cesarean section was performed in five of these because

of fetal distress. Decreased amniotic fluid was observed in all three cases of congenital anomaly.

Fundal and suprapubic pressure elicited bradycardia in four cases, all of which had decreased amniotic fluid volume. Three of these patients were delivered by cesarean section because of fetal distress in labor and all had preceding nonreactive patterns.

### Comment

With the use of the NST as the primary approach in AFHRT, a reactive NST has been considered "normal" while a nonreactive NST followed by a positive CST has been considered "abnormal."<sup>3</sup> The nonreactive NST followed by a negative CST has been considered normal although recent data from this institution suggested that this combination should be considered inconclusive and that the testing should be repeated in 24 hours.<sup>7</sup> The finding of an abnormal test (nonreactive NST and positive CST) has often led to the consideration of immediate delivery whereas a normal NST result with a reactive pattern is usually repeated in 1 week unless the clinical situation changes dramatically. The suspicious or equivocal CST following a nonreactive NST is routinely repeated in 24 hours. The preponderance of nonreactive NSTs associated with bradycardia is clear in this study. The outcome in such cases even when followed by a negative CST gives further credence to the approach which requires retesting in 24 hours.

From the data in this study, it would appear that the appearance of a prolonged bradycardia during

AHFRT, irrespective of whether the NST is reactive or nonreactive, should be considered abnormal. It would seem reasonable to consider prompt delivery in most circumstances, particularly when the fetus is mature. In such cases, a trial of labor is justified, provided that continuous fetal monitoring during labor is possible. Retesting or continuous surveillance would appear desirable in the face of fetal immaturity or when delivery is not considered feasible (Fig. 1).

Ultrasonic scanning may assist in detection of oligohydramnios and in the diagnosis of congenital anomalies. Oligohydramnios is often associated with intrauterine growth retardation of a variety of etiologies including the anomalous fetus. Abnormal FHR patterns, both antepartum<sup>8,9</sup> and intrapartum,<sup>10</sup> are more commonly seen in the abnormal fetus.

It would seem reasonable to apply fundal and suprapubic pressure at the conclusion of all tests. This procedure is relatively simple to perform, does not add significantly to the length of the test, and may detect decreased amniotic fluid and potential cord entrapment. This simple procedure when provoking bradycardia in conjunction with an abnormal AFHRT identifies a group of patients particularly susceptible to intrapartum distress.

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# Evaluation of the Corometrics 315 telemetry system for fetal monitoring

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The Corometrics 315 telemetry system is a commercially available unit which can transmit both fetal heart rate (FHR) and intrauterine or external pressure data to a standard fetal monitor. This system was evaluated in 40 patients, ten of whom were monitored simultaneously by telemetry and direct-wire fetal monitoring. Because of some differences in electronic engineering between the telemetry and direct fetal monitoring systems, the following differences were found: Comparison of the simultaneously obtained FHR recordings revealed that the telemetry system rejected most of the motion artifacts while maintaining true variability. There were no differences as regards readability, FHR, and beat-to-beat variability. The intrauterine pressure values were the same, although the telemetry system gave a smoother waveform with less motion artifact. The main advantages of the telemetry system were found to be: (1) The patient was free to move about and ambulate during labor. (2) It was possible to obtain uninterrupted monitoring while the patient was moved from the labor room to the delivery room and throughout the second stage of labor. (AM. J. OBSTET. GYNECOL. 139:199, 1981.)

DURING THE EARLY 1960s, it was shown that radio-telemetry, which is the wireless transmission of data via radio waves to a receiver, could be used to transmit the fetal heart rate (FHR).<sup>1, 2</sup> During the 1970s, the single-channel telemetry was expanded to a two-channel system whereby the second channel could be used to record intrauterine pressure.<sup>3-6</sup> Although the single-channel FHR recordings could be performed by means of commercially available equipment with minimal modifications, the transmission of FHR and intrauterine pressure required custom-built two-channel transmitters and receivers.

The introduction of the Corometrics 315 telemetry

system has made it possible to record both FHR and intrauterine or external pressure signals. The quality of recordings made by radiotelemetry with custom-made equipment has been shown to be equivalent to that of recordings obtained by direct-wire fetal monitoring.<sup>6</sup> As telemetry has several advantages, it is anticipated that the introduction of commercial equipment will see more widespread use of this method of monitoring. Because of some differences in electronic engineering between the telemetry and direct fetal monitoring systems, the first part of this study was designed to evaluate differences in recordings between the two systems when used in the same patient at the same time. The second part of the study was the evaluation of the telemetry system when used in several different clinical situations.

## Material and methods

The Corometrics 315 telemetry system consists of a transmitter, receiver, antenna, transducers, and interconnecting cables which can be used with the standard Corometrics fetal monitor. The transmission range is optimally 100 feet, by line of sight. In our building, since the signal had to go through walls and around corners, the range was limited to 60 to 80 feet. To

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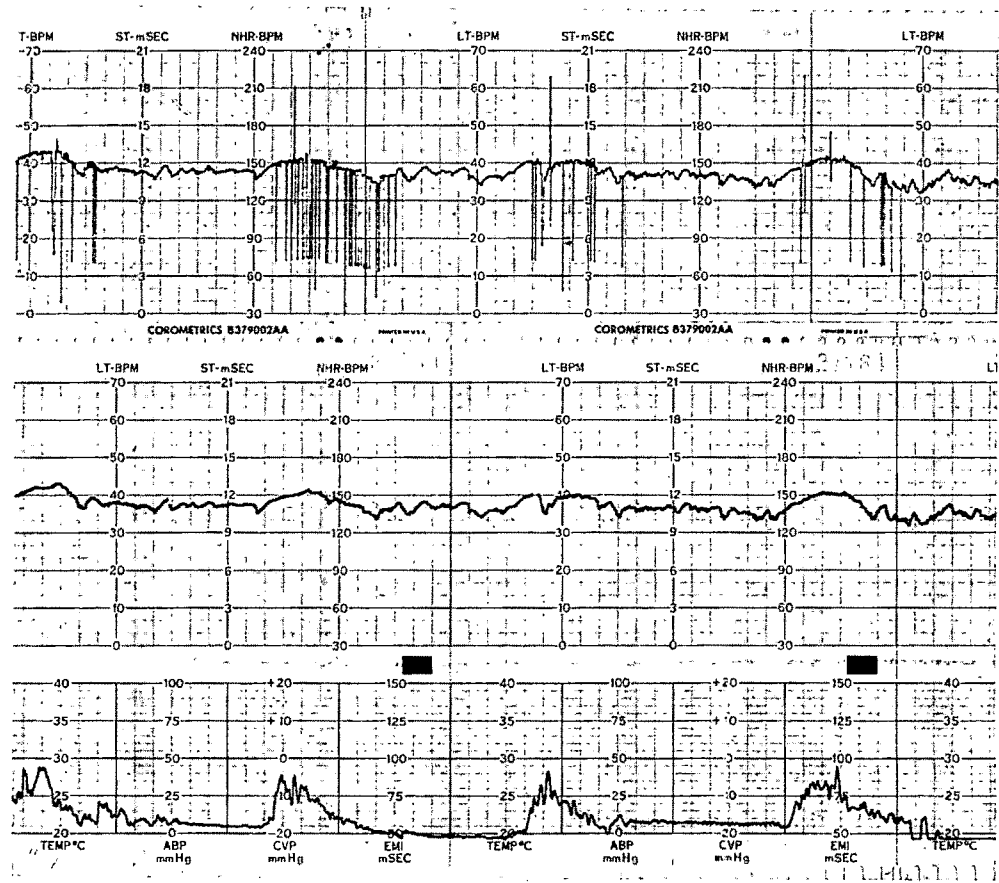


**Fig. 1.** *Upper tracing:* Direct tracing from Corometrics III monitor indicating motion artifact. *Middle tracing:* Telemetry tracing. Motion artifact rejected. True variability maintained. *Lower tracing:* Telemetry tracing of intrauterine pressure (IUP).

ensure uninterrupted signal reception over a broad area throughout the study, a Corometrics omnidirectional antenna system was installed in the suspended ceiling of the labor and delivery unit. The antenna was mounted on the underneath side of the ceiling with the cables above the ceiling. The number of antennas required is determined by the architecture of the building and the range required and not by the number of receivers.

The system operates in the Federal Communications Commission's allocated range of 176 to 214 MHz and requires standard frequency modulation (FM) techniques, i.e., varying voltage levels are converted to varying frequency changes. These changes are transmitted to the receiver where they are changed back to varying voltages and displayed.

The transmitter measures 1.7 by 3.3 by 4.9 inches and weighs 9 ounces, including one 9-volt alkaline battery. The antenna system is powered through the receiver module, which in turn is powered by the fetal monitor. The transmitter uses the transducer leads as

the transmitting antenna. The spiral electrode is virtually identical to the standard electrode, the only difference being in the lead lengths, which are 18 inches long in order to reach up to the transmitter connections. Ordinarily the transmitter is placed on the abdomen of the patient. No leg plate is necessary since the transmitter is battery powered, which provides the necessary 60 Hz noise rejection that the leg plate would ordinarily provide. The pressure cable is also about 18 inches long for the same reason as the spiral electrode. The internal catheter is shorter (40 inches long) than the standard pressure catheter since the pressure transducer is also mounted on the abdomen, next to the transmitter. This shorter length also helps to reduce motion artifact.

The frequency response of the FHR input is 1 to 70 Hz. The lower limit of 1 Hz reduces baseline wander which is generally increased when a patient is mobile. The upper limit of 70 Hz reduces excessive motion artifact, which is usually increased when the patient is mobile.



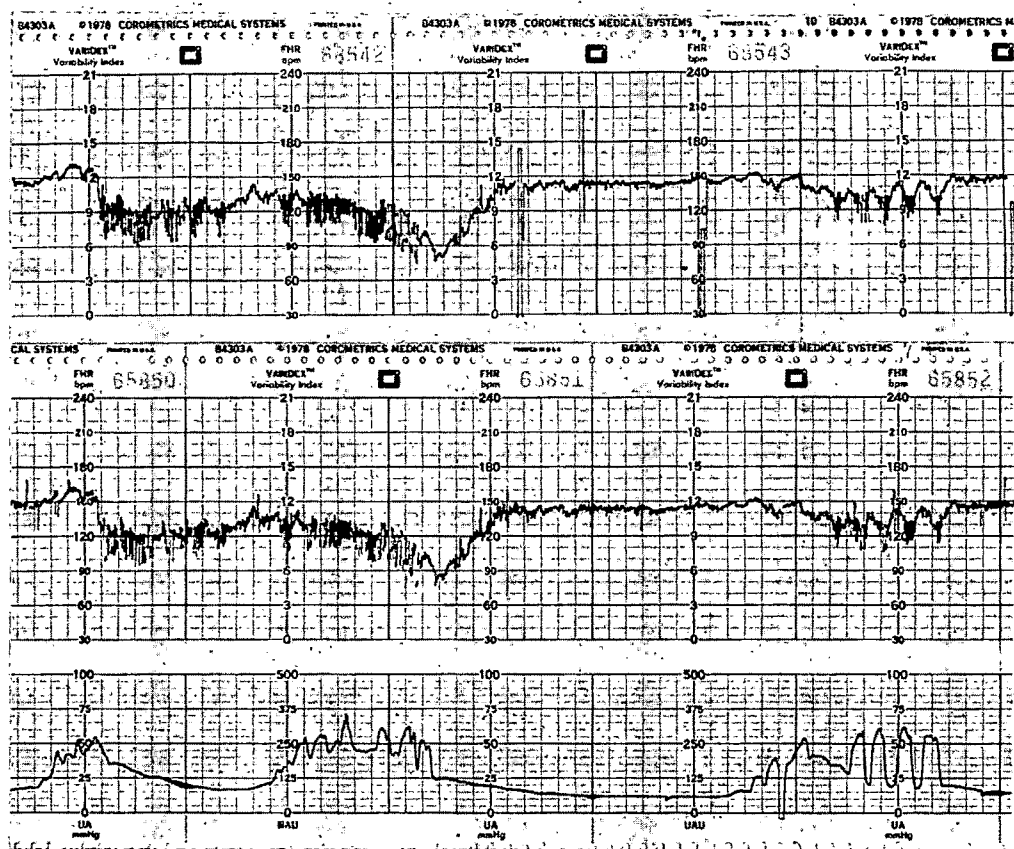


**Fig. 2.** *Upper tracing:* Direct tracing from Corometrics 112 monitor indicating apparent FHR arrhythmia. *Middle tracing:* Telemetry tracing of the same event. Arrhythmia is detected while most artifact is still rejected. *Lower tracing:* Telemetry tracing of IUP.

Both intrauterine and external pressure transducers can be used with the Corometrics system. The frequency response of the pressure channel, when the recorder is used with telemetry, is direct current (DC) to 0.6 Hz. This provides rejection of excessive artifact in the internal mode as well as rejecting most respiration artifact when a tocotransducer is used. Thumping on the catheter, as is often done as a system check, will produce only very slight pen deflection on the telemetry, usually utilizes a DC range of 10 Hz since respiration artifact is not a factor.

Forty patients were studied, 10 of whom were monitored simultaneously by telemetry and direct-wire fetal monitoring. First, the patient was monitored by the usual method, by means of a leg plate and intrauterine pressure system. After this was accomplished the telemetry was added to the system. Another set of electrode leads was connected from the leg plate to the telemetry transmitter and another catheter was attached from the regular strain gauge to the telemetry strain gauge. Then both strain gauges were rezeroed. This caused no detectable differences between pres-

sure readings, and there was no interference with the original setup. The remaining 30 patients were monitored, both while ambulatory and at bed rest, through all phases of labor including delivery by cesarean section or vaginal delivery, which included forceps delivery. Intrauterine pressure was monitored in 17 of the patients while the rest were monitored with the tocotransducer.

### Results and comment

**FHR.** A comparison of the simultaneously obtained FHR tracings showed no differences as regards readability, FHR, and beat-to-beat variability between the telemetry and standard tracings. An advantage of the telemetry tracing was the rejection of motion artifact while true variability was maintained (Fig. 1). If an arrhythmia was present, this was recorded by the telemetry system while most artifacts were still rejected (Fig. 2). No adverse fetal outcome occurred as a result of monitoring by telemetry.

**Pressure recording.** As evidenced by Fig. 3, the 0.6 Hz cutoff point does not alter the value of the pressure

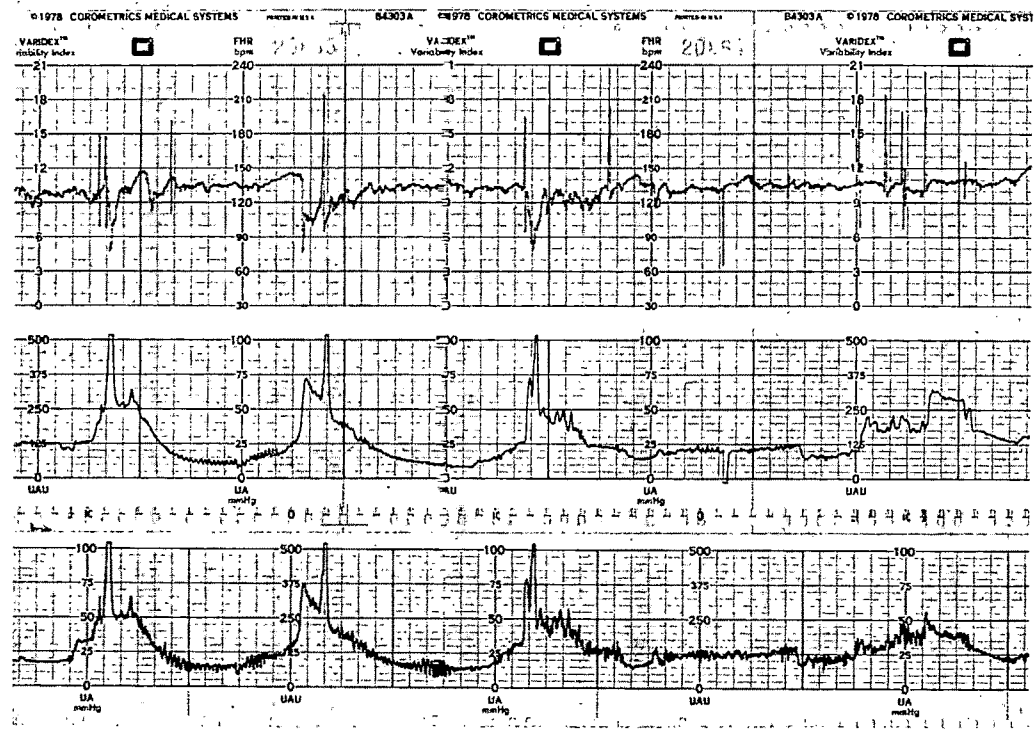


Fig. 3. Upper tracing: Telemetry FHR tracing. Middle tracing: Telemetry IUP tracing. Trace is smoother than lower direct tracing, but there is no significant loss of pressure information. Push spikes are retained. Lower tracing: Direct IUP tracing from Corometrics III monitor showing typical motion artifact present with active patient.

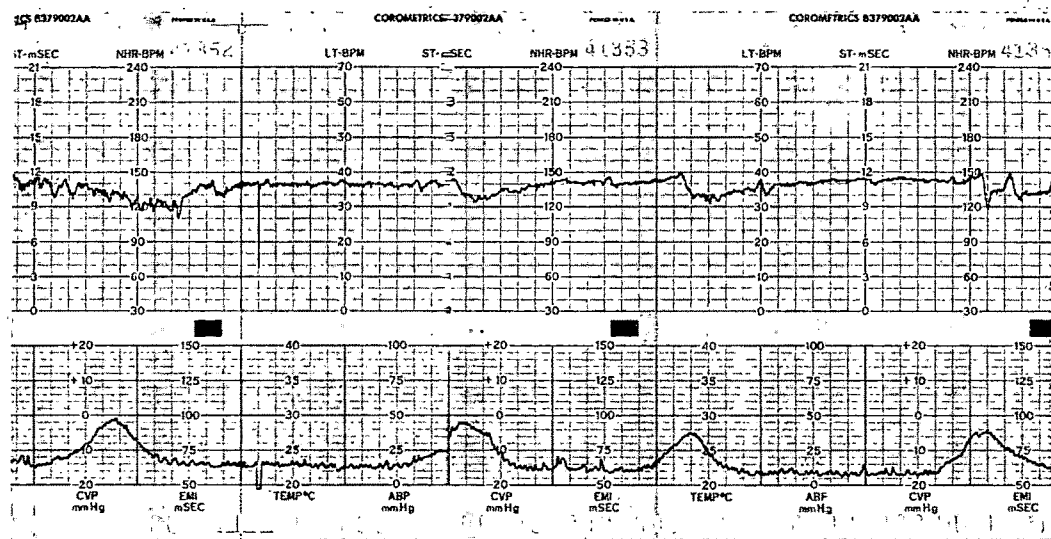


Fig. 4. Telemetry tracing recording FHR and IUP in an ambulatory patient.



reading, although it does smooth the waveform somewhat, as explained earlier.

As with direct-wire monitoring for intrauterine pressure, the baseline pressure shows some alteration when the height of the pressure transducer is changed relative to the tip of the intrauterine catheter. Thus, with telemetry, when the patient changed position from the supine to the upright, a slight increase in baseline pressure was noted.

The use of the external tocodynamometer is affected by variables such as the placement of the transducer, the intensity of the contractions, and the thickness of the abdominal wall. In the suitable ambulatory patient, after correct placement of the external tocodynamometer, telemetry proved satisfactory for the assessment of the frequency and length of uterine contractions.

In the 30 patients monitored by telemetry alone, the

system worked well, and except for the differences noted above, the recordings did not differ from those obtained by standard monitoring techniques. Two of the most important advantages of the telemetry system, as compared to standard monitoring techniques, are as follows: (1) The patient is no longer "attached" to the monitor. She can thus move freely from a lying position in bed, to sitting on a chair, to full ambulation. Even when the patient went to the toilet, the recording continued to be satisfactory. An example of the quality of the tracings obtained in ambulatory patients is shown in Fig. 4. (2) A major advantage of the telemetry system is the capability of obtaining uninterrupted monitoring while moving the patient from the labor room to the delivery room and being able to continue the FHR monitoring during the second stage of labor.

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# Catecholamines in human amniotic fluid

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This study was undertaken to determine the amniotic fluid levels of epinephrine (E), norepinephrine (N), and dopamine (D) at different gestational ages in the human. Amniotic fluid obtained from 32 pregnant women undergoing amniocentesis for medical indications was analyzed with a radioenzyme assay for these catecholamines. The gestational ages ranged from 17 to 39 weeks. No patient was in active labor at the time of amniocentesis. The data obtained in this study demonstrated a rise of all three catecholamines at the end of the third trimester:  $N = 362.7 \pm 61.2$  pg/ml ( $\pm$ SEM),  $E = 179.6 \pm 45.4$  pg/ml ( $p < 0.05$ ), and  $D = 2717.0 \pm 836.6$  pg/ml ( $p < 0.01$ ). The physiologic role for the increasing amounts of catecholamines, especially D, in amniotic fluid is unknown; however, these amines could be the stimulus for the intrauterine synthesis of prostaglandins as parturition approaches. (AM. J. OBSTET. GYNECOL. 139:204, 1981.)

THE FACT THAT catecholamines play a role in fetal development was first suggested by the observation of Zuckerkandl, in 1901, of the development of extra-adrenal chromaffin tissue in the human fetus. Multiple studies<sup>1-4</sup> since that time have confirmed the anatomic and biochemical development of this tissue; however, its physiologic role in fetal and neonatal life remains unclear. Zuspan and Abbott,<sup>5</sup> in 1970, first described the presence of catecholamines in human amniotic fluid. With the use of a trihydroxyindole (THI) fluorometric assay, they found free norepinephrine and epinephrine in a small sample of patients. Woodman and associates,<sup>6</sup> in 1978, confirmed the presence of these two amines in amniotic fluid; the norepinephrine concentration they observed was three times higher than that of epinephrine. Ben-Jonathan and Maxson,<sup>7</sup> in 1978, evaluated the changes of catecholamines in fetal rat amniotic fluid with the progression of pregnancy. These authors found a significant rise in the dopamine concentration near term. Other studies<sup>8,9</sup> have proved that physiologic levels of catecholamines do not cross the placenta in significant quantities; therefore, the interpretation of the amniotic fluid data has

been that catecholamines found in the fetal compartment are of fetal origin.

The present study was undertaken to evaluate catecholamine levels in human amniotic fluid with progression in gestation.

## Material and methods

Amniotic fluid for this study was obtained from 32 pregnant women undergoing amniocentesis for medical indications. The gestational ages based on the date of the last menstrual period and ultrasound evaluation ranged from 17 to 39 weeks. The medical indications included midtrimester pregnancy termination, placenta previa, isoimmunization, diabetes mellitus, and elective repeat cesarean section. No patient was receiving sympathomimetic drugs or any other agent known to interfere with the radioenzyme assay for catecholamines. No patient was in active labor at the time of amniocentesis. An amniotic fluid volume appropriate for gestational age was confirmed by physical examination and ultrasound evaluation. Immediately after amniocentesis, 1 ml of amniotic fluid was placed into an iced tube containing 1.2 mg of glutathione and 1.8 mg of ethylene glycol-bis ( $\beta$ -aminoethyl ether) N,N'-tetraacetic acid (EGTA). After thorough mixing, the tubes were stored at  $-70^{\circ}\text{C}$  until catecholamine assay was performed. None of the specimens utilized for this study was contaminated with blood or meconium. Catecholamine assay was performed within 3 weeks of obtaining amniotic fluid samples.

The catecholamine assay was performed with a radioenzymatic assay (Cat A Kit, Upjohn Diagnostic) according to the basic technique described by Passon and Peuler.<sup>10</sup> Aliquots of 50  $\mu\text{l}$  of amniotic fluid were

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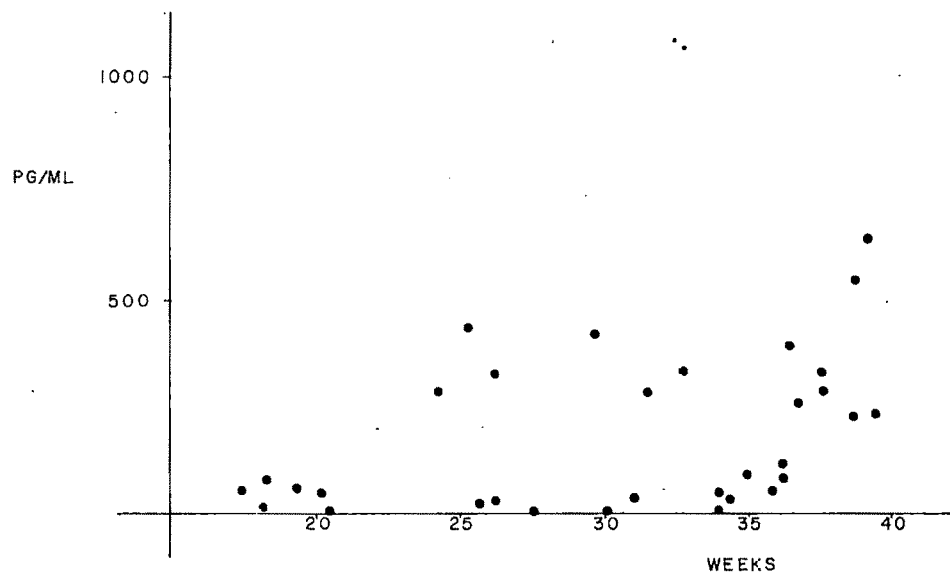


Fig. 1. Distribution of amniotic fluid norepinephrine concentration in picograms per milliliter in human amniotic fluid from 17 to 39 weeks of gestation.

assayed in duplicate. With the utilization of catechol-o-methyl transferase (COMT) and tritiated S-adenosyl-l-methionine ( $^3\text{H}$ -SAM) in a buffered solution, the catecholamines were simultaneously converted to their meta  $^3\text{H}$ -methoxy derivatives. To prevent the conversion of 3,4-dihydroxyphenyl-alanine (DOPA) into dopamine, benzylhydroxyamine hydrochloride was added to the COMT preparation. After extraction into toluene/isoamyl alcohol (3:2, v:v), then back into 0.1M acetic acid, the catecholamine derivatives were separated by thin-layer chromatography with silica gel GF, 250  $\mu$ , 10 by 20 cm prescored plates (Analtech Uniplate) in a tertiary amyl alcohol:toluene:methylamine solution (6:2:2, v:v:v). The derivatives were identified under an ultraviolet light source (254 nm wavelength), then scraped into individual liquid-scintillation vials.  $^3\text{H}$ -methoxytyramine (dopamine derivative) was eluted into 0.05M ammonium hydroxide, then extracted into toluene:isoamyl alcohol:Liquifluor\* (700:300:50, v:v:v).  $^3\text{H}$ -metanephrine (epinephrine derivative) and  $^3\text{H}$ -normetanephrine (norepinephrine derivative) were eluted into 0.05M ammonium hydroxide. These derivatives were oxidized to  $^3\text{H}$ -vanillins with the use of a 4% sodium metaperiodate solution. After the oxidation reaction was stopped with glycerol and the solution was acidified with 0.1M acetic acid, the  $^3\text{H}$ -vanillins were extracted into a toluene:Liquifluor (1,000:50, v:v) solution. Samples were counted in a liquid-scintillation counter (Searle Mark II liquid scintillation counter).

Assay sensitivity is 2 to 5 pg/50  $\mu\text{l}$  sample for

\*New England Nuclear.

norepinephrine and epinephrine and 15 to 20 pg/50  $\mu\text{l}$  sample for dopamine. The interassay coefficients of variation were 19% for dopamine, 16% for epinephrine, and 12% for norepinephrine. Samples were grouped into four gestational categories: 17 to 20 weeks, 21 to 32 weeks, 33 to 36 weeks, and 37 to 39 weeks. The mean  $\pm$  SEM values for these four gestational categories were then determined. Unpaired t test analysis (two-tailed) was utilized to determine statistical significance between the 21- to 32-week group and the later gestations.

## Results

The individual norepinephrine results are demonstrated in Fig. 1. The data were grouped into gestational categories, and the mean  $\pm$  SEM values were: 17 to 20 weeks' gestation,  $49.8 \pm 11.9$  pg/ml; 21 to 32 weeks' gestation,  $196.9 \pm 51.8$  pg/ml; 33 to 36 weeks' gestation,  $107.4 \pm 42.8$  pg/ml; and 37 to 39 weeks' gestation,  $362.7 \pm 61.2$  pg/ml. As demonstrated, the norepinephrine concentration in amniotic fluid remained low throughout gestation, increasing somewhat at term.

The individual epinephrine values are demonstrated in Fig. 2. The grouped mean  $\pm$  SEM results are as follows: 17 to 20 weeks' gestation,  $35.8 \pm 13.9$  pg/ml; 21 to 32 weeks' gestation,  $82.2 \pm 20.3$  pg/ml; 33 to 36 weeks' gestation,  $69.5 \pm 10.6$  pg/ml; and 37 to 39 weeks' gestation,  $179.6 \pm 45.4$  pg/ml. Similar to the norepinephrine levels, the epinephrine concentrations in amniotic fluid were extremely low and remained so until a significant ( $p < 0.05$ ) rise at term occurred.

Similar to the other two catecholamines measured in

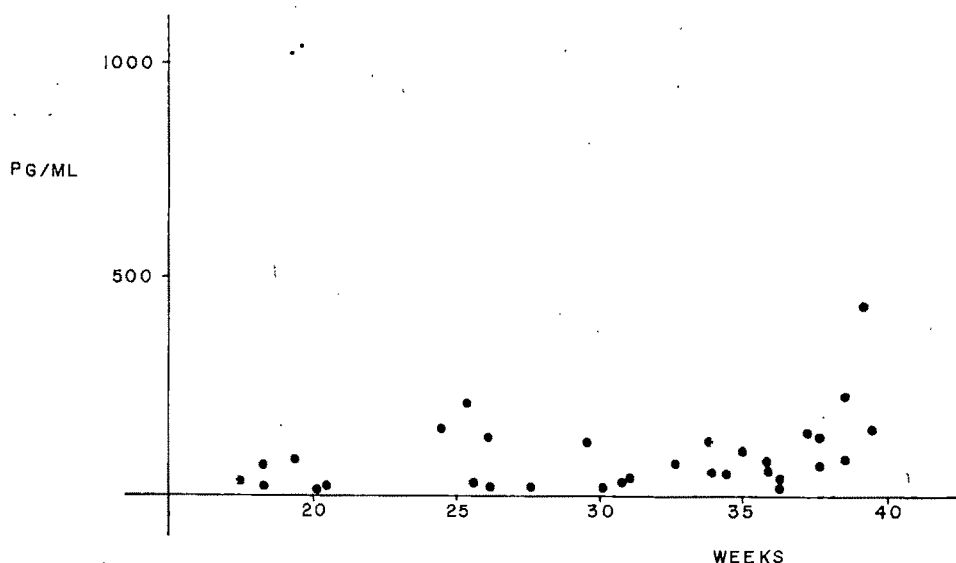


Fig. 2. Distribution of amniotic fluid epinephrine concentration in picograms per milliliter in human amniotic fluid from 17 to 39 weeks of gestation.

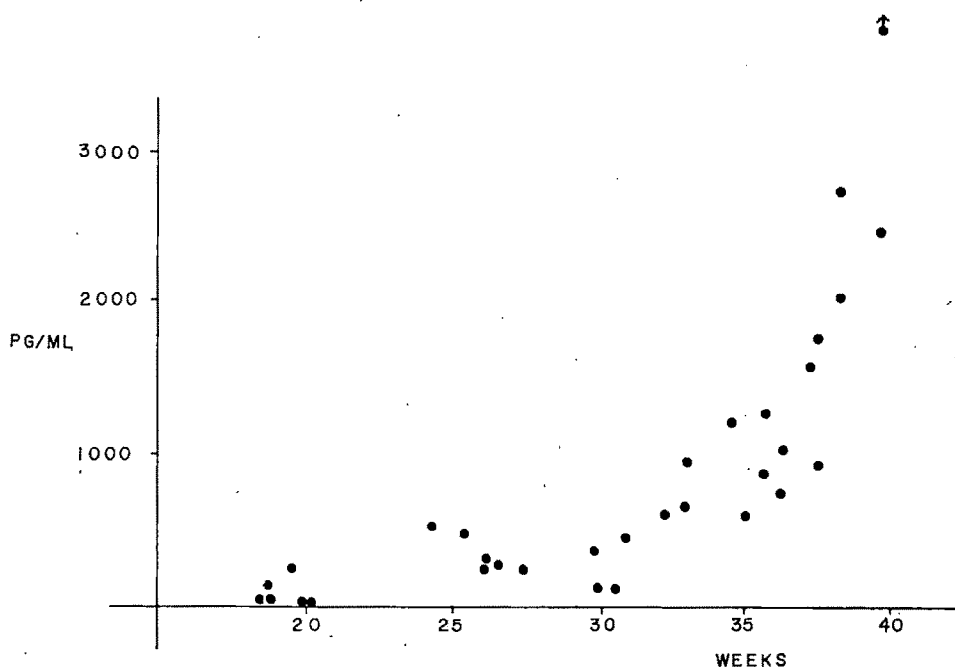


Fig. 3. Distribution of amniotic fluid dopamine concentration in picograms per milliliter in human amniotic fluid from 17 to 39 weeks of gestation. Highest dopamine value was 7,568 pg/ml at 39 weeks of gestation.

this study, dopamine levels were extremely low in the group at 17 to 20 weeks' gestation (Fig. 3). The dopamine levels, however, then increased significantly throughout gestation: 21 to 32 weeks' gestation,  $337.1 \pm 48.1$  pg/ml; 33 to 36 weeks' gestation,  $905.8 \pm 81.1$  pg/ml ( $p < 0.01$ ); and 37 to 39 weeks' gestation,  $2,717.0 \pm 836.6$  pg/ml ( $p < 0.01$ ). Comparison of dopamine to norepinephrine and epinephrine (Fig. 4) demonstrates

significantly more dopamine than the other two catecholamines at 33 to 36 weeks' gestation ( $p < 0.001$ ) and 37 to 39 weeks' gestation ( $p < 0.02$ ).

#### Comment

Much of the early anatomic and histochemical work<sup>1-4</sup> on fetal chromaffin tissue confirmed that this tissue developed significantly during fetal life, then de-



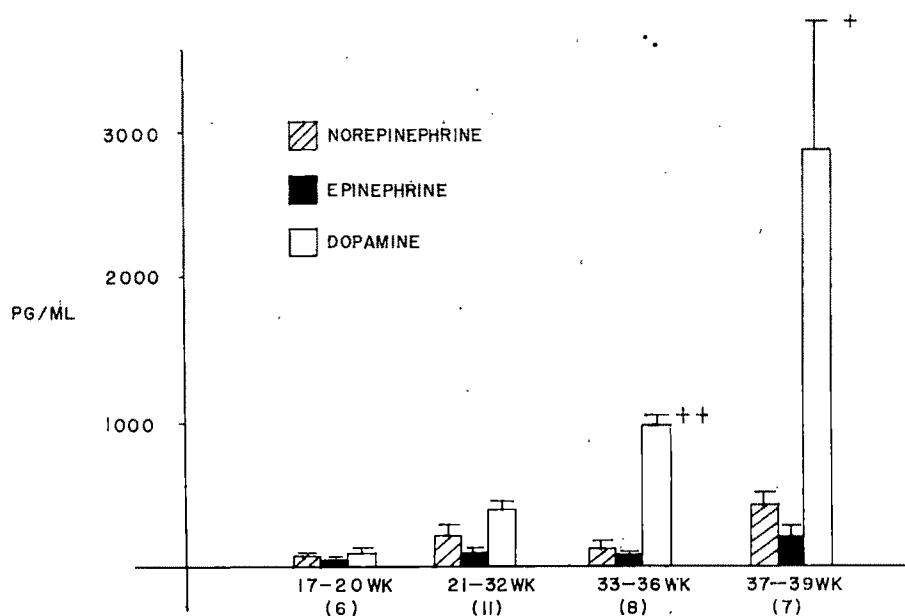


**Fig. 4:** Amniotic fluid catecholamine levels (mean  $\pm$  SEM) for four gestational groups: 17 to 20 weeks, 21 to 32 weeks, 33 to 36 weeks, and 37 to 39 weeks. Number in parentheses represents number of patients sampled in each group. + =  $p < 0.02$ , and ++ =  $p < 0.001$  for comparison of dopamine value to the other two catecholamine values in each gestational group.

generated during early childhood. In an extensive review of the extra-adrenal chromaffin tissue, Coupland<sup>4</sup> demonstrated that the organ of Zuckerkandl, the largest of the para-aortic chromaffin bodies, which is located at the level of the inferior mesenteric artery, reaches peak maturation at term. Coupland also demonstrated cellular maturation of these organs from primitive sympathetic cells to functional pheochromocytes during fetal life. West and associates,<sup>3</sup> in 1951, with a bioassay for catecholamines made one of the early attempts to evaluate the catecholamine content of fetal chromaffin tissue. These authors demonstrated a progressive increase in amine content, mainly norepinephrine, in the organ of Zuckerkandl which peaked during the peripartum period. Greenberg and Lind<sup>2</sup> likewise found higher catecholamine levels per gram of tissue in the human fetal para-aortic chromaffin bodies than in any other major organ in the body, including the adrenal. DeGallardo and associates<sup>1</sup> measured norepinephrine, epinephrine, and dopamine in the organ of Zuckerkandl in the rat. In that study norepinephrine and dopamine levels were found to be higher than those of epinephrine.

Fetal chromaffin functional activity was first definitely demonstrated by Comline and Silver,<sup>11</sup> in 1966, in which they demonstrated that hypoxia mainly produced a discharge of norepinephrine in the fetal lamb and calf. Jones and Robinson,<sup>8</sup> in 1975, demonstrated that the fetal lamb rapidly responded to hypoxia with a predominant output of norepinephrine. Artal and as-

sociates,<sup>12</sup> in 1979, measured the fetal lamb catecholamine response to maternal hemorrhage. The highest elevation of catecholamines in their study occurred in norepinephrine, and the least was in dopamine. A study done in our laboratory<sup>13</sup> on the fetal rat response to acute hypoglycemia likewise demonstrated a significant rise in fetal catecholamines.

Functional studies in human subjects have been performed only at the time of delivery. Lagercrantz and associates<sup>15</sup> found catecholamine levels to be significantly higher in newborn plasma than in plasma of resting adults. Newborn catecholamine levels in their study were even higher in complicated deliveries such as breech presentation and deliveries complicated by birth asphyxia. Blouquit and associates<sup>16</sup> likewise found higher plasma catecholamine levels in complicated deliveries.

Amniotic fluid in late pregnancy is composed mainly of fetal urine. Approximately 1% to 5% of injected catecholamine is excreted as active hormone into the urine.<sup>14, 17</sup> Early neonatal urine has been found to contain significant quantities of epinephrine and norepinephrine; dopamine was not measured.<sup>14</sup> Zuspan and Abbott<sup>5</sup> were the first to demonstrate free catecholamines in human amniotic fluid. Woodman and associates<sup>6</sup> utilized a radioenzyme assay to measure amniotic fluid epinephrine and norepinephrine levels in 50 pregnancies near term (37 to 43 weeks' gestation). The mean norepinephrine level found in their study was 8.1 nmoles/L (1,370 pg/ml) and the mean epinephrine

level was 2.6 nmoles/L (476 pg/ml); dopamine was not measured. The amniotic fluid catecholamine levels measured in our study showed a significant rise in all three catecholamines near term, most significantly in dopamine. The mean catecholamine levels in the 37- to 39-week group in our study were: norepinephrine, 362.7 pg/ml; epinephrine, 179.6 pg/ml; and dopamine, 2,717.0 pg/ml. The difference between our near-term levels and those of Woodman and co-workers can probably be explained by the fact that their study included pregnancies that had progressed to 43 weeks. The increasing trend demonstrated in our study would suggest that these later gestations would have even higher catecholamine levels.

The predominance of dopamine in amniotic fluid has previously been observed by Ben-Jonathan and Maxson.<sup>7</sup> These authors measured rat amniotic fluid catecholamines with a radioenzyme assay and found that amniotic fluid dopamine levels increased significantly, peaking at parturition, whereas levels of norepinephrine and epinephrine remained relatively stable. The physiologic role of this increasing quantity

of dopamine is unknown. Some authors<sup>18, 19</sup> have demonstrated that dopamine effects are mediated through prostaglandin synthesis and/or release.

Prostaglandin levels in the amniotic fluid and uterus have been demonstrated to increase with increasing gestational age.<sup>20, 21</sup> The stimulus for the increase in prostaglandins is unknown. Tai and associates<sup>22</sup> have shown in studies in vitro that epinephrine, norepinephrine, and dopamine result in a four to five time increase in prostaglandin E<sub>2</sub> and F<sub>2</sub>α synthesis by microsomes from the renal medulla. Utilizing a similar assay in vitro, Falkay and associates<sup>23</sup> demonstrated that epinephrine produces a dose-related increase in prostaglandin E<sub>2</sub> and F<sub>2</sub>α synthesis by microsomes in human myometrium during pregnancy. From these observations one could formulate the theory that the increasing amniotic fluid catecholamine levels, especially those of dopamine, are a fetal signal that facilitates intrauterine prostaglandin production and the eventual onset of parturition. In conclusion, this study begins to suggest a functional role for the transient chromaffin development that occurs in fetal life.

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# Comparison of mercury levels in maternal blood, fetal cord blood, and placental tissues

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Previous studies have reported that mercury accumulates in cord blood during pregnancy. This study was carried out to determine where in cord blood the mercury accumulates, i.e., in cord erythrocytes, in cord plasma, or in both, and to determine whether the predominant form of mercury which accumulates is methyl or inorganic mercury. From our data it is clear that methyl mercury accumulates in cord erythrocytes: A total of 30% more methyl mercury was found in fetal erythrocytes than in maternal erythrocytes. Also correlation analysis of the methyl mercury levels in maternal and fetal erythrocytes showed a strong correlation ( $r = 0.87$ ). In regard to inorganic mercury, the highest concentration was found in the placenta, suggesting a barrier role, but a significant correlation ( $r = 0.62$ ) was also found between the maternal and fetal plasma levels of inorganic mercury. Moreover, the inorganic mercury concentration per gram of plasma was higher in fetal cord plasma than in maternal plasma. Overall, the relative levels of methyl and inorganic mercury reported here varied considerably in maternal and fetal erythrocytes, plasma, and in the placenta, but all of the levels were low ( $<6$  ng Hg/gm of tissue) and in agreement with "total" mercury levels reported by others. (AM. J. OBSTET. GYNECOL. 139:209, 1981.)

THERE ARE SEVERAL reports of "normal" mercury concentrations in human fetal and maternal blood at birth.<sup>1-5</sup> These reports show that the average mercury concentration in cord blood is 20% to 30% greater than the corresponding mercury concentration in maternal blood. They do not show, however, whether this accumulation occurs in the red blood cells, in the plasma, or in both.

Moreover, the chemical form of mercury which accumulates in cord blood is unknown. In the above studies the total amount of mercury in blood, rather than the amount of organic and inorganic mercury, was re-

ported. This was done because suitable methods for distinguishing the forms at "normal" levels of exposure have not been available. Interestingly, inferential data from animal and human studies suggest that the principal form which accumulates is methyl mercury, the most toxic form of mercury.<sup>1, 6</sup>

The purpose of this investigation was to use a recently developed gas chromatography technique to determine accurately the concentration of methyl and inorganic mercury in maternal and fetal cord blood and in the placentas of a normally exposed patient population. In addition, our purpose was to analyze the red blood cells and plasma separately for methyl and inorganic mercury.

## Methods

**Patients and sample collection.** Twenty-nine pregnant women, placentas, and fetuses (matched) were included in this study. The mothers resided in or very near Cleveland, Ohio, and were delivered in Cleveland Metropolitan General Hospital. They were not selected in regard to prenatal or intrapartum medical problems, although clinical information was recorded in depth. Informed consent was obtained from the subjects in the appropriate manner.

Maternal and cord blood samples were obtained in

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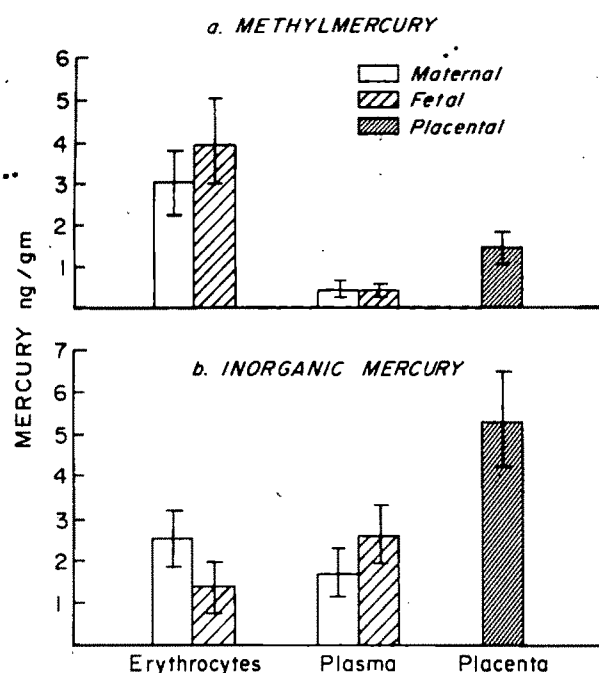


Fig. 1. Relative levels of mercury in maternal and fetal blood and placenta.

heparinized tubes; placental samples were placed in polyethylene containers. For maternal blood analysis, 6 ml of venous blood was drawn when the mother was admitted to the hospital in labor. For fetal blood analysis, 6 ml of venous umbilical cord blood was obtained immediately following delivery of the infant. Also collected at delivery was a 6 gm aliquot of a perfused placental sample; the placenta was perfused with a sucrose buffer. All of the samples were stored frozen until analyzed for mercury.

#### Determination of methyl and inorganic mercury.

The blood and placental samples were prepared for mercury analysis by means of alkaline digestion. A sample of 2 gm was placed in a 20 ml glass scintillation vial, and 2 ml of 45% sodium hydroxide and 1 ml of 1% cysteine hydrochloric acid were added. The contents were heated until dissolved; boiling was avoided. The mixture was cooled and then diluted to a known final volume.

Following alkaline digestion, the mercury was extracted according to a modification of the method reported by Cappon and Smith.<sup>7</sup> It was modified to eliminate the need for radioisotopes and to permit the analysis of low levels of mercury in blood.<sup>8</sup> This was accomplished by a peak height ratio technique with ethyl mercury chloride used as an internal standard; ultrapure solvents, acids, and Teflon Oak Ridge tubes were also utilized. Glassware and the gas chromatograph

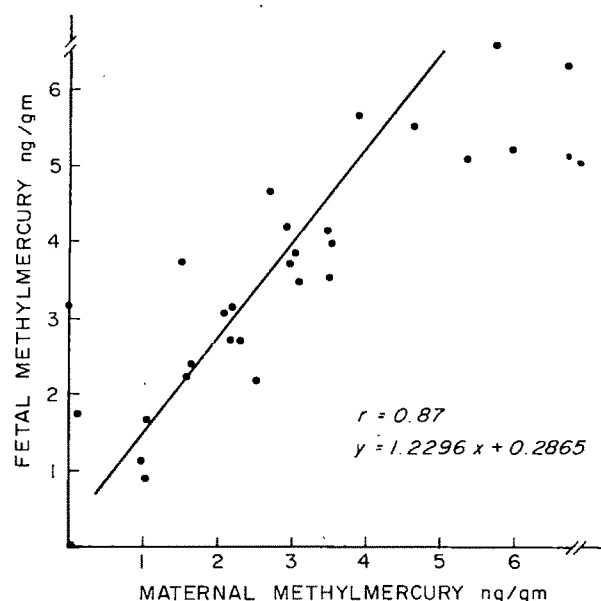


Fig. 2. Correlation between maternal and fetal erythrocyte methyl mercury concentrations.

graph inlet, detector, and top of column were kept clean. Any remaining inorganic mercury contamination was compensated for by using extracted standards and peak height ratio standard curves.

Quantitative analysis of the organic and inorganic mercury was accomplished with a Hewlett-Packard 5840 A gas chromatograph equipped with a  $\text{Ni}^{63}$  electron capture detector. Chromatographic separation was accomplished with the use of a 4-foot, 3% ECNSSM AW dimethylchlorosilane (DMCS)-treated glass chromatography column treated daily with DMCS.

The sensitivity, precision, and repeatability of the method have been discussed elsewhere.<sup>7, 8</sup> All of the samples were run in duplicate.

**Data analysis.** Statistical analyses were performed with a Hewlett-Packard Model 41C programmable calculator and the paired t test and linear regression programs described in the instruction manual.

#### Results

Fig. 1, *a* and *b*, illustrates the relative levels of methyl and inorganic mercury found in maternal and fetal blood and in the placenta. The erythrocyte and plasma levels are reported separately. The data show that the highest concentration of methyl mercury was found in the erythrocytes, as compared to plasma and placental tissue (Fig. 1, *a*); the least amount was found in the plasma. In addition, the fetal erythrocytes had more methyl mercury than the maternal erythrocytes. Fig. 1, *b*, shows that the highest concentration of inorganic mercury was found in the placenta—twice as much as



Table I. Comparison of methyl and inorganic mercury levels in maternal and fetal blood and placenta

| Tissue        | Methyl mercury*<br>(ng/gm) | Inorganic mercury*<br>(ng/gm) | Ratio of methyl to<br>inorganic mercury | Level of<br>significance |
|---------------|----------------------------|-------------------------------|-----------------------------------------|--------------------------|
| Erythrocytes: |                            |                               |                                         |                          |
| Maternal      | 3.0 ± 2.1 (29†)            | 2.5 ± 1.8 (28)                | 1.2                                     | NS                       |
| Fetal         | 3.9 ± 3.0 (29)             | 1.4 ± 1.2 (28)                | 2.8                                     | p < 0.001                |
| Plasma:       |                            |                               |                                         |                          |
| Maternal      | 0.4 ± 0.2 (25)             | 1.7 ± 1.4 (22)                | 0.2                                     | p < 0.001                |
| Fetal         | 0.4 ± 0.3 (25)             | 2.6 ± 1.9 (22)                | 0.2                                     | p < 0.001                |
| Placenta      | 1.4 ± 1.1 (24)             | 5.3 ± 3.2 (24)                | 0.3                                     | p < 0.001                |

NS: Not significant.  
\*Mean ± SD  
†Number of samples.

Table II. Comparison of maternal and fetal blood levels of methyl and inorganic mercury

| Tissue       | Form of mercury | Maternal (ng/gm) | Fetal (ng/gm) | % Difference | Level of significance |
|--------------|-----------------|------------------|---------------|--------------|-----------------------|
| Erythrocytes | Methyl          | 3.0 ± 2.1        | 3.9 ± 3.0     | +30          | p < 0.01              |
|              | Inorganic       | 2.5 ± 1.8        | 1.4 ± 1.2     | -44          | p < 0.01              |
| Plasma       | Methyl          | 0.4 ± 0.2        | 0.4 ± 0.3     | 0            | NS                    |
|              | Inorganic       | 1.7 ± 1.4        | 2.6 ± 1.9     | +53          | p < 0.02              |

NS: Not significant.

Table III. Correlation between the levels of methyl and inorganic mercury in maternal and fetal blood and placenta

| Form of mercury | Factors correlated                       | N  | r    | P     |
|-----------------|------------------------------------------|----|------|-------|
| Methyl          | Maternal versus cord red blood cells     | 29 | 0.87 | <0.01 |
|                 | Maternal red blood cells versus placenta | 24 | 0.55 | <0.05 |
|                 | Cord red blood cells versus placenta     | 24 | 0.56 | <0.05 |
|                 | Maternal versus cord plasma              | 25 | 0.42 | NS    |
|                 | Maternal plasma versus placenta          | 22 | 0.17 | NS    |
|                 | Cord plasma versus placenta              | 22 | 0.22 | NS    |
| Inorganic       | Maternal versus cord red blood cells     | 24 | 0.14 | NS    |
|                 | Maternal red blood cells versus placenta | 23 | 0.01 | NS    |
|                 | Cord red blood cells versus placenta     | 23 | 0.1  | NS    |
|                 | Maternal versus cord plasma              | 22 | 0.62 | <0.01 |
|                 | Maternal plasma versus placenta          | 21 | 0.06 | NS    |
|                 | Cord plasma versus placenta              | 21 | 0.22 | NS    |

NS: Not significant.

was found in the other tissues. Also a greater amount of inorganic mercury was found in fetal plasma than in fetal erythrocytes, while the converse was true for maternal erythrocytes and plasma. All of the tissues studied had low mean (<6 ng Hg/gm of tissue) concentrations of methyl and inorganic mercury.

In Table I the levels of methyl and inorganic mercury in the tissues studied are compared. No statistically significant difference was observed between the levels of methyl and inorganic mercury in maternal erythrocytes, but a significant difference was observed between the two forms of mercury in fetal erythrocytes. Moreover, it can be seen by using a ratio of methyl to inorganic mercury that almost three times more methyl

mercury than inorganic mercury was found in fetal erythrocytes. In contrast the maternal and fetal plasma and the placenta had significantly more inorganic mercury than methyl mercury. The ratio of methyl mercury to inorganic mercury was less than 0.3 for the maternal and fetal plasma and the placenta.

A comparison between maternal and fetal erythrocyte and plasma levels of methyl and inorganic mercury is shown in Table II. Significantly more methyl mercury (p < 0.01) was found in fetal erythrocytes than in maternal erythrocytes; this difference was approximately 30%. In addition, the fetal erythrocytes contained significantly less inorganic mercury than the maternal erythrocytes. When the maternal and fetal

plasma levels of mercury were compared, fetal plasma was found to contain significantly more inorganic mercury than maternal plasma, while the levels of methyl mercury were found to be approximately equal.

Correlation analysis was performed on the various tissue samples analyzed for mercury; the results are shown in Table III. In regard to methyl mercury, the most significant correlation was between maternal and cord red blood cells ( $r = 0.87$ ,  $p < 0.01$ ). Also significant were the correlations between maternal red blood cells and placenta and between cord red blood cells and placenta. However, it is important to note that the level of methyl mercury found in the placenta was very low (Fig. 1). Comparison of the inorganic mercury levels of the various tissues revealed a significant correlation between those of maternal plasma and cord plasma ( $r = 0.62$ ,  $p < 0.01$ ). Additional correlations were determined between the levels of methyl and inorganic mercury in the same tissues; none of these was statistically significant.

The most striking correlation noted in Table I was between the methyl mercury levels in maternal and fetal red blood cells. A scatterplot of this relationship is shown in Fig. 2. It is interesting to note that for a given level of methyl mercury in maternal red blood cells the corresponding level in fetal red blood cells is higher. Also, in two cases, methyl mercury was measurable in fetal but not in maternal red blood cells.

### Comment

On the basis of experimental and epidemiologic data, Berglund and associates<sup>9</sup> concluded that the most reliable index of methyl mercury exposure is the level of methyl mercury in red blood cells. This level reflects the retention of methyl mercury in the body as well as in the nervous system.<sup>9</sup> Thus, the red blood cell data presented here are a good index of the exposure of our subjects to methyl mercury in our particular environment. There are, unfortunately, no human studies similar to ours with which our red blood cell methyl mercury data may be compared.

Our data on methyl and inorganic mercury in erythrocytes and plasma can, however, be converted to whole-blood "total" mercury levels and then compared with several previous studies. This may be done by using the following formula:

$$\text{HgB} = \text{Ht} (\text{Ce}) + (1 - \text{Ht}) \text{Cp}$$

where HgB equals the "total" mercury concentration in blood; Ht equals the hematocrit; Ce equals the concentration of mercury in erythrocytes (organic plus inorganic); and Cp equals the concentration of mercury in plasma (organic plus inorganic). We calculated the "to-

tal" mercury concentration and found maternal and fetal cord blood, respectively, to have 3.4 and 3.9 ng Hg/gm of tissue. Others have reported values of 8.7 and 11.5 ng/gm,<sup>4</sup> 1 and 1.3 ng/gm,<sup>1</sup> 6 and 7.7 ng/gm,<sup>5</sup> and 16.8 and 20 ng/gm,<sup>2</sup> for maternal and fetal cord blood, respectively. These results are all from normally exposed populations of pregnant women and fetuses; the variability is apparently due to diet, environmental factors, and analytical methodology. All of these studies and the present one confirm that the "total" (organic plus inorganic) mercury levels in fetal cord blood are 13% to 24% higher than those found in maternal blood.

It is also interesting to compare our maternal and cord blood data in terms of methyl mercury in whole blood (excluding inorganic mercury). Using the above formula, we found the maternal and fetal methyl mercury whole-blood levels, respectively, to be 1.4 and 2.0 ng/gm of tissue, that is, 32% more methyl mercury was present in fetal cord whole blood. This is similar to the 30% increase we observed when the methyl mercury levels in maternal and fetal packed red blood cells were compared. Thus, regardless of whether the data are expressed in terms of nanograms of methyl mercury per gram of packed red blood cells or in whole blood, significantly more methyl mercury is present in fetal cord blood.

Previous investigators have reported significant correlations between mercury levels in maternal and fetal cord blood. Baglan and associates<sup>4</sup> and Roels and associates<sup>10</sup> reported correlation coefficients of 0.71 and 0.62, respectively. These studies differed from the present study in that they measured and correlated the "total" mercury content of maternal and fetal cord blood. In the present study, methyl mercury and inorganic mercury were analyzed separately in both erythrocytes and plasma. Our data show a striking correlation between the erythrocyte methyl mercury levels of the mother and fetus ( $r = 0.87$ ); this is in agreement with the high level of permeability of the placenta to methyl mercury, as reported by others.<sup>1, 2, 6</sup> Our data also show a strong correlation between the plasma levels of inorganic mercury ( $r = 0.62$ ), suggesting that inorganic mercury also passes through the placenta.

The concentration of mercury in the placenta has been found to vary considerably. Mean levels as high as 71.5 ng Hg/gm of tissue have been reported in Japan<sup>2</sup> and mean levels as low as 2.3 ng Hg/gm of tissue have been reported in Iowa.<sup>1</sup> Moreover, considerable variation has been reported when placental samples were collected from three locations and analyzed in the same laboratory: Placental samples were obtained from patients in Charlotte, North Carolina, Birmingham,



Alabama, and Augusta, Georgia, and their mean mercury levels ranged from 8 to 19 ng/gm of tissue.<sup>11</sup> The variability of the mercury levels reported in these studies is of course due to environmental factors and dietary habits, as well as sample handling and analytical techniques. For example, in this study,<sup>11</sup> elevated mercury levels in placentas that were not perfused (to eliminate blood contamination) prior to mercury analysis were also reported. In the present study, with the use of perfused placental samples, gas chromatography analysis, and samples obtained from an urban patient population, the placental "total" mercury concentration (methyl plus inorganic) was found to be 6.7 ng/gm of tissue.

The excess mercury in fetal blood has been postulated as being due to: (1) differences in hematocrit and (2) differences in hemoglobin binding characteristics.<sup>12</sup> But the present study shows that despite correction for the hematocrit, fetal blood contains significantly more mercury than maternal blood. In regard to binding characteristics of mercury to fetal and adult hemoglobin, White and Rothstein<sup>13</sup> were unable to find any dif-

ferences and suggested that plasma be studied more carefully in this connection. Also, Ishihara<sup>14</sup> examined this latter postulation from a theoretical point of view and pointed out that the number of thiol groups capable of binding mercury in adult hemoglobin is greater than that in fetal hemoglobin. Thus, differences in hematocrit and in hemoglobin binding do not explain the increased amount of mercury in fetal blood in the present study.

Sporadic outbreaks of methyl mercury poisoning have occurred throughout the world and involved pregnant women and newborn infants.<sup>6</sup> Questions have naturally arisen concerning the transport and distribution of mercury in the maternal-fetoplacental unit. The studies presented here describe the differential accumulation of methyl and inorganic mercury in maternal and fetal erythrocytes, plasma, and placental tissue at "natural" levels of exposure. Additional studies are needed to explain this phenomenon and to determine if the interrelationships remain the same at higher levels of exposure.

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# The effect of cervical/vaginal secretions on measurements of lecithin/sphingomyelin ratio and optical density at 650 nm

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Free-flowing amniotic fluid collected vaginally can be used in a reliable way for determination of fetal pulmonary maturity. Lavaging the vaginal/cervical area with sterile saline and examining the lavage fluid for lecithin/sphingomyelin (L/S) spots showed no detectable spots in the supernatants (one exception) and barely detectable L/S spots in the sediment. Vaginal-cervical saline-wash fluids did not affect amniotic fluid L/S ratios. Lavaging the vaginal-cervical area with abdominal amniotic fluid did not affect the L/S ratio of the original amniotic fluid. (AM. J. OBSTET. GYNECOL. 139:214, 1981.)

LECITHIN/SPHINGOMYELIN (L/S) ratio analyses of amniotic fluids collected transabdominally is now a well-established procedure for the determination of fetal pulmonary maturity. L/S ratios above 2.0 generally indicate pulmonary maturity, whereas ratios below 2.0 generally indicate pulmonary immaturity.<sup>1, 2</sup>

This test has gained universal acceptance; however, the relatively sophisticated methodology required to perform the test has limited its use. Generally, the test is not available on a 24-hour basis, 7 days a week, and often the test is never offered in the small community hospital. As an alternate to the L/S ratio, a simple, quick and accurate test that can be performed in any

laboratory at any time has recently been proposed. It has been reported that amniotic fluid optical density at 650 nm correlates with L/S ratios. Optical density readings equal to or greater than 0.15 correspond to L/S ratios of 2.0 or greater; optical density readings below 0.15 generally correlate with L/S ratios that are below 2.0.<sup>3-12</sup>

Frequently in patients with ruptured membranes, a free flow of amniotic fluid into the vagina occurs, and this may often be the only fluid available for study. Kulovich and associates<sup>13</sup> have reported that a close correlation exists between amniotic fluid L/S ratios collected by amniocentesis and L/S ratios in amniotic fluid flowing freely through ruptured membranes into the vagina. This finding was reported as a statement and no data were presented.

In an effort to establish whether vaginal and cervical secretions can interfere with amniotic fluid L/S ratios, vaginal washes were examined for L/S ratios and for their ability to interfere with L/S ratios of amniotic fluid collected transabdominally. Further, the effect of lavaging the vaginal-cervical area with amniotic fluid collected transabdominally on the L/S ratio and optical density of the recovered amniotic fluid wash will be reported.

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**Table I.** L/S ratios of vaginal-cervical fluids\*

| Gestational week | Supernatant | Pellet |
|------------------|-------------|--------|
| 10               | —           | 1:1    |
| 10               | 1:1         | 1:1    |
| 17               | —           | 1:1    |
| 18               | —           | 1:1    |
| 20               | —           | 1:1    |
| 30               | —           | 1:1    |
| 36               | —           | 1.5:1  |
| 37               | —           | 1.5:1  |
| 37.5             | —           | 1:1    |
| 39               | —           | 1:1    |

\*Approximately 9.0 ml of vaginal-cervical wash fluids was centrifuged at 2,000 xg for 10 minutes. Then 3.0 ml of supernatant and the pellet resuspended in 2.0 ml of saline were analyzed for L/S ratios.

**Material and methods**

Sterile normal saline (10 ml) was used to gently wash the vagina and cervix of 10 pregnant patients of gestational ages of 10 to 40 weeks. In an additional six patients, approximately 10 ml of the patients' transabdominally collected amniotic fluid of known L/S ratio and optical density was gently introduced into the vagina and cervix for lavaging. Approximately 80% to 90% of these fluids was recovered and analyzed for L/S ratios and optical density measurements.

All fluids were centrifuged within 1 hour at 2,000 x g for 10 minutes. The supernatants from the saline washes were analyzed for inherent L/S ratios and for their effect on known amniotic fluid L/S ratios. The pellets from the saline washes were resuspended in 2.0 ml of saline and the entire amount was used for L/S ratio determination. L/S ratios and optical density readings at 650 nm were done as previously described.<sup>5</sup> Optical density readings of the 2,000 x g supernatant fluid were obtained in a 1 cm light path cuvette against distilled water at 650 nm in a Beckman DB spectrophotometer. L/S ratios were measured with a commercial kit (Chromato Screen Analysis Kit, TLC Corp., Miami, Florida).

To study the possible effect of the saline vaginal-cervical wash supernatants on known amniotic fluid L/S ratios, an aliquot of different supernatants was added to different amniotic fluids of known L/S ratios. The resulting fluids were then analyzed for L/S ratios and compared with the amniotic fluids diluted with saline as controls.

**Results**

It is apparent from Table I that nine out of the 10 vaginal-cervical supernatant fluids tested did not have detectable L/S ratios. One sample had faint but detectable L/S at a ratio of 1:1. The pellets from all of these

**Table II.** Effect of vaginal cervical washings on amniotic fluid L/S ratios

| L/S ratios    |                     |               |
|---------------|---------------------|---------------|
| Untreated AF* | AF and supernatant† | AF and saline |
| 2.8           | 2.5                 | 2.5           |
| 3.7           | 3.4                 | 3.6           |
| 2.7           | 2.3                 | 2.5           |
| 1.3           | 1.1                 | —             |

\*Amniotic fluid.

†Vaginal-cervical wash was centrifuged at 2,000 xg for 10 minutes. Then 1.0 ml of supernatant or 1.0 of saline and 1.0 ml of AF were combined and L/S ratio was determined.

**Table III.** L/S ratios and optical density at 650 nm of amniotic fluid collected transabdominally and subsequently lavaged in vaginal-cervical area\*

| Patient No. | L/S     |           | Optical density at 650 nm |           |
|-------------|---------|-----------|---------------------------|-----------|
|             | Vaginal | Abdominal | Vaginal                   | Abdominal |
| 1           | 2.3     | 2.5       | 0.27                      | 0.23      |
| 2           | 3.8     | 3.6       | 0.70                      | 0.68      |
| 3           | 3.6     | 3.8       | 0.41                      | 0.69      |
| 4           | 3.4     | 3.8       | 0.60                      | 1.4       |
| 5           | 1.8     | 1.6       | 0.05                      | 0.05      |
| 6           | 2.5     | 2.4       | 0.92                      | 0.77      |
| 7           | 2.2     | 2.2       | —                         | —         |
| 8           | 1.3     | 1.6       | —                         | —         |

\*Approximately 10 ml of transabdominally collected amniotic fluid was introduced into the vaginal-cervical area. L/S ratios and optical density readings were obtained on the resulting lavage and transabdominally collected amniotic fluid after centrifugation at 2,000 xg for 10 minutes.

2,000 x g centrifuged vaginal-cervical wash fluids had L/S ratios of 1:1 except two with ratios of 1.5:1.

In an effort to learn the possible effect of vaginal-cervical supernatant wash fluid on amniotic fluid with a known L/S ratio, an equal volume of wash fluid and amniotic fluid was combined and analyzed. The results in Table II indicate that there is no significant effect of the supernatant fluid on the L/S ratio of untreated amniotic fluid. Dilution with saline, as to be expected, also did not significantly affect the untreated amniotic fluid L/S ratio.

The effect of lavaging the vaginal-cervical area with transabdominally collected amniotic fluid on the L/S ratio and the optical density at 650 nm was studied and the results are presented in Table III. The L/S ratios of the vaginal-cervical lavages and the transabdominally collected fluids are not significantly different. The optical density of six of the lavaged fluids was also measured and all of the readings were indicative of pulmonary maturity.

### Comment

The effect of vaginal-cervical secretion on amniotic fluid L/S ratios is an important consideration when transabdominal amniocentesis cannot be performed and only vaginally collected amniotic fluid is available. To the best of our knowledge, no studies specifically directed at determining whether vaginal-cervical secretions have detectable quantities of L/S have been reported. The presence of significant quantities of either lecithin or sphingomyelin or both in these secretions would make unsatisfactory amniotic fluid collected in this way. Further interaction of these secretions with known amniotic fluid surfactants would similarly make unsatisfactory vaginally collected amniotic fluids.

In this study, we have demonstrated that no detectable quantities of either surfactant are present in the vaginal-cervical washes (Table I). It must be pointed out, however, that before study these washings were centrifuged at  $2,000 \times g$  for 10 minutes. This centrifugation speed and time are the same as those we routinely use in determining amniotic fluid L/S ratios and readings of amniotic fluid optical density at 650 nm. In one of ten samples tested, a faint but detectable L/S ratio was observed. The low amount of L/S in this fluid would not be expected to interfere with a fluid collected vaginally. The pellets in all cases had detectable

L/S ratios; in fact, in all cases, an additional unidentified surfactant was present. Since all amniotic fluid samples collected vaginally or at amniocentesis are centrifuged at  $2,000 \times g$  for 10 minutes before examination, these substances would be removed; thus, no interference is expected.

The possibility that supernatants of vaginal-cervical washings may interact with known amniotic fluid L/S ratios was studied and from the results noted it would appear that no such interaction is present (Table II). The fact that the ratios were not significantly altered is not unexpected, as dilution should not affect a ratio. Finally, directly lavaging the vaginal-cervical area with transabdominally collected amniotic fluid does not appear to significantly change the L/S ratio or the optical density of the amniotic fluid at 650 nm (Table III).

From the above, it would appear that free-flowing amniotic fluid collected vaginally can be used in a reliable way for determination of fetal pulmonary maturity. In a subsequent publication, we will report the results obtained with amniotic fluid of patients with premature rupture of the membranes, collected both transabdominally and vaginally.

We wish to thank Pat Christopharo for the procurement of test samples and Roberta Millham for the preparation of this manuscript.

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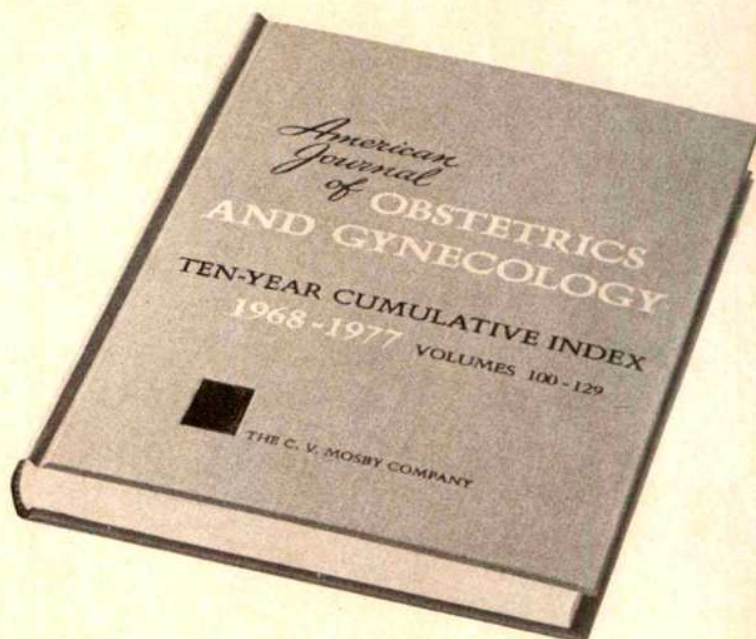
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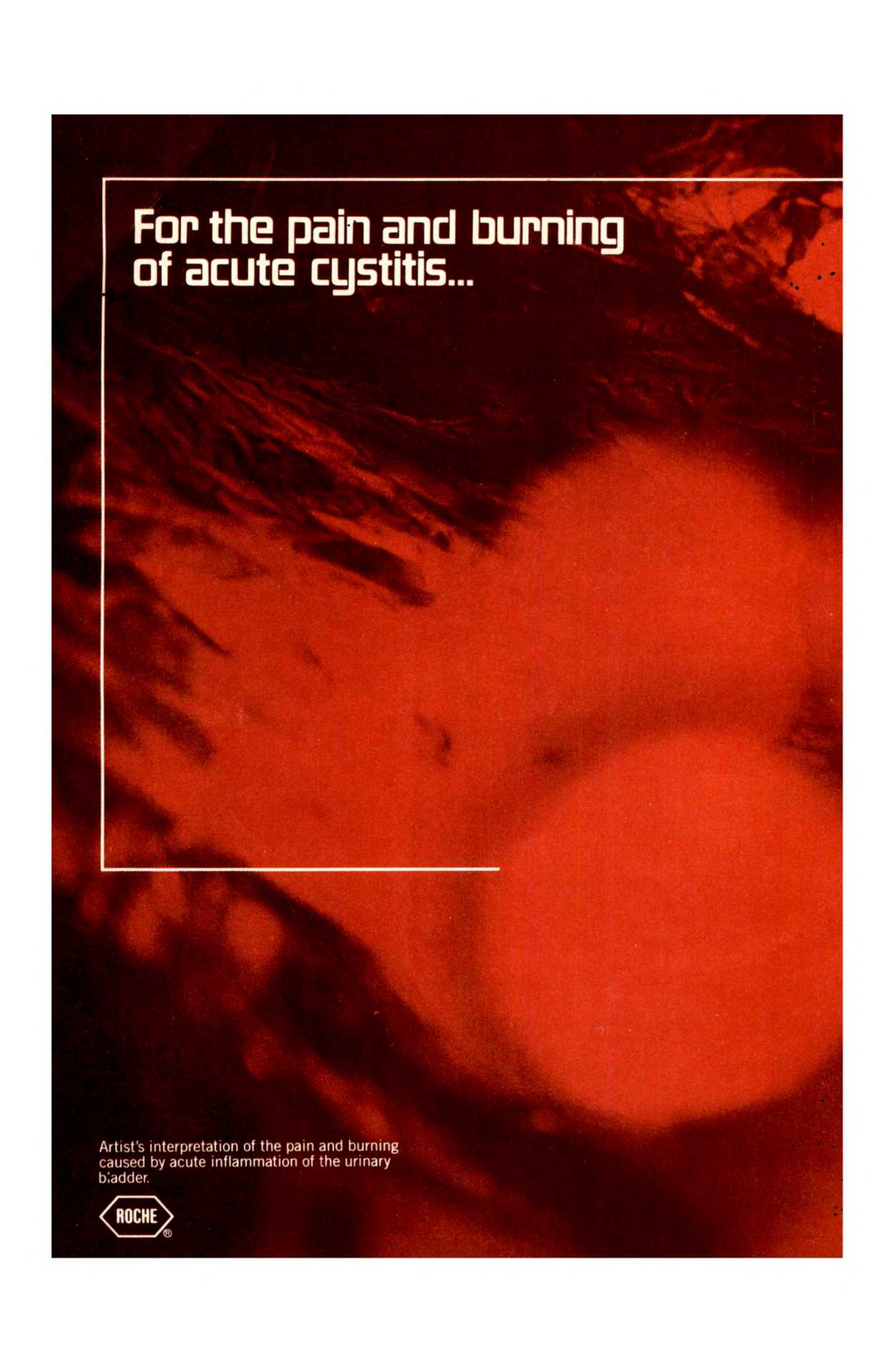
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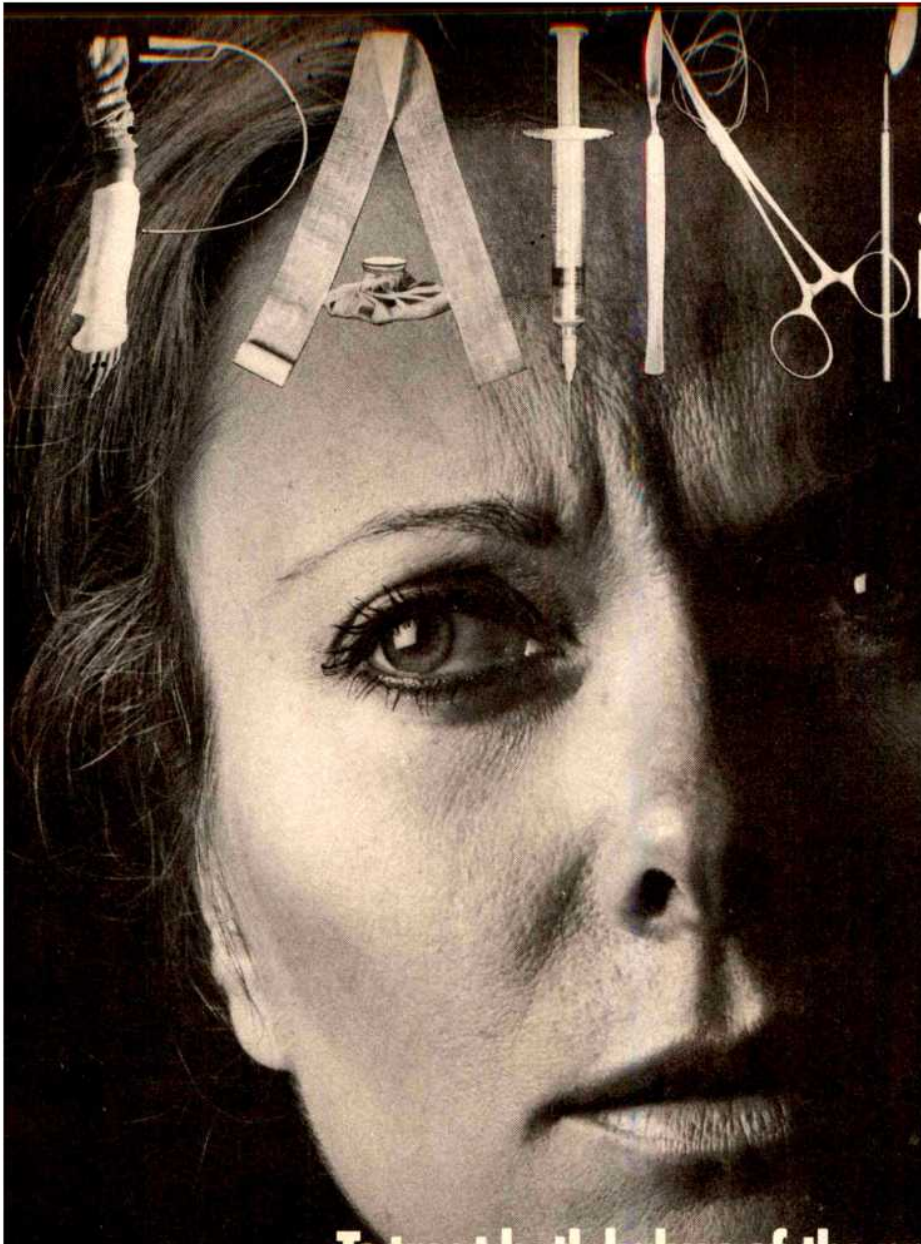
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**Usage in Children:** Since there is no experience in children who have received this drug, safety and efficacy in children have not been established.

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Promethazine should be administered cautiously to patients with cardiovascular or liver disease. Synalgos-DC should be given with caution to certain patients such as the elderly or debilitated, and those with hypothyroidism, Addison's disease, and prostatic hypertrophy and urethral stricture.

**ADVERSE REACTIONS:** The most frequently observed

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**DOSAGE AND ADMINISTRATION:** Dosage should be adjusted according to the severity of the pain and the response of the patient. Synalgos-DC is given orally. The usual adult dose is two capsules every 4 hours as needed for pain.

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### Gastrointestinal hemorrhage in puerperium of pre-eclamptic patients who received glucocorticoid therapy

STEFAN SEMCHYSHYN, M.D.

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GASTROINTESTINAL HEMORRHAGE due to ulcerations has been reported in preeclamptic patients,<sup>1</sup> and this condition has been described in one patient on steroid therapy.<sup>2</sup> However, to my knowledge, hemorrhage in association with steroidal therapy in pre-eclamptic patients has not been reported.

In Case 1, D. E., a 29-year-old Filipino woman, gravida 1, para 0, presented for delivery on September 9, 1973, at 38 weeks' gestation. Her pregnancy was unremarkable until 8 weeks prior to admission, at which time she developed peripheral edema. This was treated with hydrochlorothiazide. On admission, she complained of marked swelling, headaches, and nervousness. The blood pressure was 140/100 torr. Optic fundi were normal, and the chest was clear. Abdominal size was appropriate for the length of gestation. The fetus was engaged in a cephalic presentation. Urinalysis revealed proteinuria (+3). Hematologic parameters were all normal. She was sedated overnight and labor was induced in the morning. Because of a diminishing output of urine, she underwent cesarean section and was delivered of a male infant. The Apgar score was 8 at 1 and 5 minutes, and the baby weighed 2,830 grams. Post partum, while receiving antibiotic therapy, she developed hypothermia (35° C). Therapy for the hypothermia included hydrocortisone, 500 mg every 6 hours. She subsequently developed an abscess in the right lower quadrant of the abdomen which was drained through the lower abdominal incision. Her temperature remained stable; however, she developed coffee-ground vomitus, which rapidly progressed to

heavy, bright red gastrointestinal hemorrhage. Despite massive transfusions (6 U of packed cells, 8 U of whole blood, and 2 U of fresh frozen plasma over 26 hours), she did not respond to replacement therapy. Gastroscopy revealed acute erosive gastritis and duodenitis. On the following day, a total gastrectomy and side-to-side proximal jejunoesophagostomy were performed. The surgical specimen showed that the stomach was studded with numerous mucosal ulcers. The patient's postoperative course was uneventful. She subsequently had another pregnancy which was uncomplicated.

In Case 2, P. S., a 19-year-old white woman, gravida 2, para 1, presented in February, 1979, at 36 weeks' gestation with an increased gain in weight, hypertension, proteinuria, and epigastric pain of 1 day's duration. Physical examination showed a blood pressure of 150/110 torr. Funduscopic examination disclosed segmental vasospasm of the retinal vessels. The initial laboratory data revealed hemoconcentration, and urinalysis showed proteinuria (3+). Fetal lung immaturity was documented by analysis of amniotic fluid. Initial therapy consisted of the intravenous administration of magnesium sulfate to prevent seizures. Hydralazine was used to control the blood pressure. In order to enhance pulmonary lung maturation, the patient was given hydrocortisone prior to delivery. Delivery was effected by repeat cesarean section; the infant weighed 3 pounds 14 ounces and had Apgar scores of 6 and 8 at 1 and 5 minutes. Twenty-four hours postoperatively, the patient developed abdominal distention, and large amounts of coffee-ground material were drained by nasogastric tube. Despite lavage with ice-cold saline solution and the administration of an antacid, the patient developed gastrointestinal hemorrhage. The hemoglobin of 8.9 gm/dl was raised by 3 U of packed cells to 10 gm/dl. During the subsequent 3 days, she underwent marked diuresis up to 10,050 ml/24 hours, while receiving only 1,750 ml in the same 24-hour period. Bleeding recurred on the fourth postoperative day, at which time gastroscopy was performed and multiple ulcerations on the lesser curvature of the stomach were observed. Conservative therapy, together with cimetidine, 300 mg every 6 hours, in addition to replacement of blood and fluids was carried out. The hypertension was treated with alpha-methyldopa. The baby had an uneventful course. She responded favorably.

The association of peptic ulcers and pregnancy is not uncommon; however, symptoms of ulceration rarely

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develop for the first time during normal pregnancy. Although the issue of whether pregnancy affords protection against peptic ulcers is not settled, some reports do indicate that increased estrogen, increased histaminase, and a decreased volume of gastric secretions during pregnancy may be protective. The ulcerogenic effects of steroidal therapy are well known. Severe stress, such as burns, pre-eclampsia, or surgical procedures, may cause stress ulcers of the gastrointestinal mucosa. Indeed, in Case 1, sepsis, in addition to pre-eclampsia, could possibly have played an important role. Gastric hemorrhage, although an uncommon condition complicating pregnancy, causes not only a diagnostic dilemma, but also a therapeutic one. During pregnancy, gastrointestinal examination without the use of radiography is preferable because of the teratogenic and carcinogenic potential of radiography. Insofar as treatment is concerned, it depends on a number of factors, such as the severity of hemorrhage, presence of perforation or penetration, and the stage of gestation. In the first case, surgical therapy (although radical) resulted in a good outcome. Medical therapy was effective in the second case, with the patient benefiting from the administration of cimetidine. Since the discovery of a more effective medical agent, this mode of therapy has become more appealing and ought to be tried whenever possible.

In order to enhance fetal lung maturation in women giving birth prematurely, one may use glucocorticoids to reduce the risk of hyaline membrane disease; but caution is advised since the pre-eclamptic patient may be at increased risk of gastrointestinal hemorrhage if glucocorticoid therapy is used.

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### Gonococcal peritonitis following uterine manipulation at laparoscopy

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MANIPULATION OF THE UTERUS for enhancement of visualization of the pelvic viscera at laparoscopy at

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The Ohio State University Hospitals is routinely provided by the insertion of a No. 15 Hegar cervical dilator, as suggested by others.<sup>1</sup> Endometrial biopsy is usually performed at the same time as the placement of the cervical dilator. The purpose of this article is to illustrate a potential hazard of this technique of manipulation of the uterus.

D. E., 20 years old, gravida 2, para 2, presented for laparoscopic evaluation of chronic pelvic pain. The patient had experienced several years of gradually increasing chronic pelvic pain and deep dyspareunia. The past medical history was remarkable for appendicitis but not for pelvic inflammatory disease. During outpatient evaluation, the white blood cell count and sedimentation rate were normal, and the physical examination showed a tender uterus but no other abnormalities. Outpatient therapy with analgesics was unsuccessful. The pelvic pain was thought to be due, most likely, to pelvic adhesions, but laparoscopy was warranted in order to establish the diagnosis. The patient underwent Hasson laparoscopy with the use of a No. 15 Hegar cervical dilator fixed by ligature to a single-tooth tenaculum attached to the anterior lip of the cervix as the instrument for manipulation of the uterus. At operation, the pelvic structures were seen to be entirely normal. The patient was discharged the following day with no apparent complications. However, on the day after discharge, she returned to the emergency room with the symptoms and signs of generalized peritonitis. The temperature was 101° F, and the white blood cell count was 27,700/cu mm with a left shift. The physical examination disclosed generalized abdominal tenderness, rigidity, and guarding. X-ray films showed subdiaphragmatic air consistent with immediate postoperative intra-abdominal air or a ruptured viscus. After the initiation of broad-spectrum antibiotic coverage, the patient underwent exploratory laparotomy through an incision extending from the xiphoid process to the symphysis pubis. At laparotomy, a large amount of purulent, foul-smelling peritoneal fluid was observed throughout the peritoneal cavity. However, the odor was not feculent and no bowel perforation was found. The uterus, ovaries, and fallopian tubes were normal and showed no evidence of acute inflammation. No further operative intervention beyond exploration was performed. On the second postoperative day, the patient defervesced. Antibiotic therapy was continued for 6 days. Cervical, peritoneal fluid, and blood cultures grew *Neisseria gonorrhoeae*. The uterine biopsy specimen showed a normal proliferative endometrium. The patient left the hospital on the seventh postoperative day against medical advice, on oral antibiotics.

Manipulation of the uterus has been shown to be advantageous during diagnostic laparoscopy in order to enhance visualization of pelvic structures. Among the other methods available, a uterine sound or Hegar dilator may be used for this purpose.<sup>1</sup> Although not generally recognized as a possible complication of laparoscopy, the invasion of the uterine cavity for manipulation could displace material from the cervix and uterus into the peritoneal cavity. Peritonitis is recognized as a potential complication of uterine curettage.<sup>2</sup> Thus, any subclinical infection in the cervix or endometrium could lead to a generalized peritonitis.

Noninvasive uterine manipulators could prevent this complication but may not afford adequate maneu-



verability of the uterus. With the high incidence of gonorrhea in our society, cultures of the cervix should be shown to be negative prior to laparoscopy if invasive uterine manipulators are to be used.

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### Detrusor muscle instability—A complication of surgery?

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NUMEROUS surgical techniques are available to treat genuine stress incontinence, while surgical failure is generally due to incorrect diagnosis, poor tissues, or inappropriate technique. Although the incidence of detrusor muscle instability after abdominal or vaginal hysterectomy seems not to be increased,<sup>1</sup> Cardozo and associates<sup>2</sup> report an 18.5% incidence of detrusor muscle instability after a Burch-type colposuspension for genuine stress incontinence in patients in whom pre-operative urodynamic studies demonstrated a stable bladder. This report describes two cases of detrusor muscle instability in order to illustrate that this disorder may complicate other operations for genuine stress incontinence.

In Case 1, the patient, 39 years old, complained of stress incontinence without any other urinary symptoms. Urodynamic investigation demonstrated incontinence on coughing without alteration in detrusor muscle pressure. A Marshall-Marchetti-Krantz operation was performed and the patient was allowed to return home after 10 days, free of urinary symptoms. Six weeks later, she complained of urinary frequency and urgency, nocturia, urge incontinence, and stress incontinence. The urine was sterile and there was no residuum. Pressure-flow studies showed a rise in detrusor muscle pressure on filling of 45 mm Hg, with incontinence. There was no evidence of obstruction to outflow.

In Case 2, the patient, 57 years old, complained of stress incontinence and urinary frequency and urgency, despite an earlier anterior colporrhaphy and bladder buttress operation. Urodynamic examination demonstrated a stable bladder, and an Aldridge sling procedure was performed. Although she was free of symptoms at 3 months, she gradually developed urinary frequency and urgency, nocturia, urge incontinence,

and stress incontinence. Urodynamic screening at 6 months showed a rise in detrusor muscle pressure on filling of 30 mm Hg, with incontinence. There was neither sphincter weakness, residual urine, nor obstruction to outflow.

The cause of the detrusor muscle instability in these two cases and in those reported by Cardozo and associates<sup>2</sup> is unclear. It was due neither to obstruction to outflow nor infection, but one may speculate that damage to the nerve supply of the bladder occurred during dissection. Meanwhile, both patients have been successfully treated by bladder training.

These reports suggest that, if urinary incontinence persists or recurs after surgical procedures for genuine incontinence, the diagnosis of detrusor muscle instability should be considered.

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### Solitary paraganglioma of the vagina—Report of a case

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PARAGANGLIOMAS (extra-adrenal pheochromocytomas) of the female genital tract are distinctly uncommon neoplasms. Presented is a case of nonfunctional vaginal paraganglioma.

A 22-year-old black woman, gravida 4, para 4, entered the hospital for the removal of "a lump in the vagina" discovered after her last delivery in April, 1979. Her past medical history and the family history were noncontributory, and there was no history of hypertension in the family. Also, monitoring of the patient's blood pressure at her past deliveries had not shown unusual bouts of hypertension. No café-au-lait spots were found, and various mucosal surfaces did not show unusual areas of pigmentation or masses.

A mass, 3 by 2.5 by 1.5 cm, was found and removed from the anterior vaginal wall close to the introitus. The mass did not involve the urinary bladder and was easily "shelled out." After the pathologic diagnosis had been made, a search for other associated neoplasms (i.e., medullary carcinoma of the thyroid) or multifocality was carried out but produced negative findings.

Pathologic examination showed a nonencapsulated tumor

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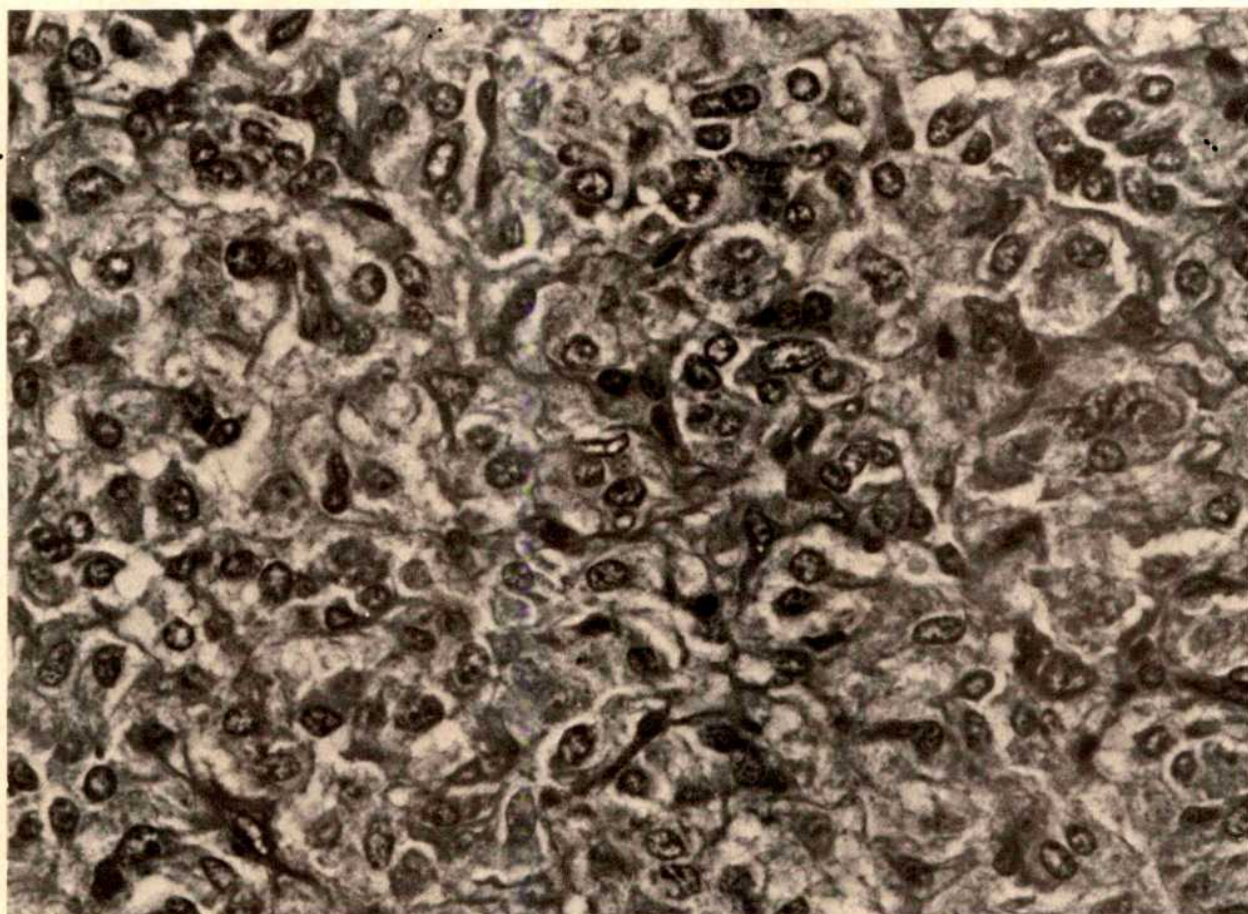


**Fig. 1.** The tumor has an organoid pattern. The tumor cells show minimal nuclear variation and ample cytoplasm. (Hematoxylin and eosin.  $\times 1,160$ .)

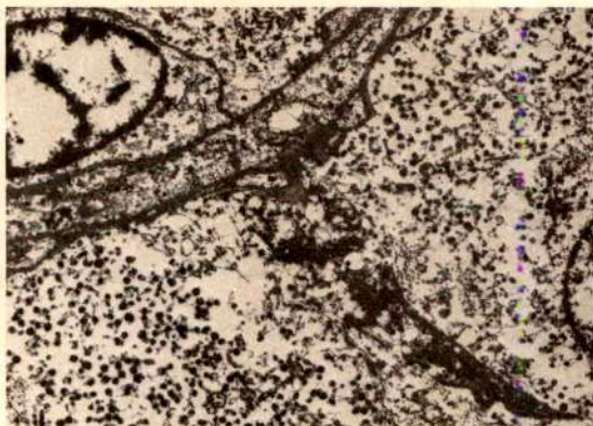


**Fig. 2.** Numerous neurosecretory granules are seen within the cytoplasm of the tumor cell. ( $\times 4,500$ .)

composed of cells with ample granular cytoplasm, small nuclei, and a few prominent nucleoli arranged in an organoid pattern (Fig. 1). Cellular atypia was minimal, and mitoses were not found. Although a positive chromaffin reaction was not observed, the Sevier-Munger neural tissue stain was strongly

positive, thereby indicating the presence of numerous argenta-ffin granules. Portions of the tumor were postfixied in osmium tetroxide and studied by electron microscopy. Large numbers of neurosecretory granules were found (Fig. 2).

Pheochromocytomas are tumors of the chromaffin system. The chromaffin bodies include the adrenal gland and the organs of Zuckerkandl. Today these cells are believed to arise from the neural crest. Approximately 10% of pheochromocytomas are extra-adrenal and are called paragangliomas.

Fries and Chamberlin,<sup>2</sup> in their review of the literature up to 1968, found only 205 documented cases of paragangliomas. They refer to one case report by Plate, in 1955, of a 66-year-old woman who presented with purulent discharge and was found to have two separate nodules of paraganglioma in the vagina. The tumors were nonfunctional. Fawcett and Kimbell<sup>1</sup> reported an ovarian paraganglioma in a 15-year-old girl who had signs of pre-eclamptic toxemia of pregnancy.

The present case seems to be the second one reported in the literature on vaginal paraganglioma, and the third instance of such a tumor in the female genital tract. That the neoplasm in the present case was a



paraganglioma is attested to by the positive argentaffin reaction and the presence of neurosecretory granules within the tumor cells. Chromaffin reaction has proved to be of limited use in these cases, and we agree with others that the prefix "nonchromaffin" should be eliminated from the terminology used to describe these tumors.

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### Unsuspected trisomy 18: A case for an examination protocol in stillborn infants

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CHROMOSOMAL abnormalities cause a wide range of phenotypic effects, sometimes making them difficult to diagnose, particularly in a stillborn premature infant. We report the case of a stillborn premature infant with trisomy 18 without any dysmorphic features whose only clinically detectable malformation was a diaphragmatic hernia. The diagnosis would probably not have been made in the absence of a protocol for the evaluation of stillborn infants which includes karyotyping.

A stillborn female infant, weighing 1,350 grams, was born to a 31-year-old Caucasian mother, gravida 2, para 0, therapeutic abortions 1, after a gestation of 34 to 35 weeks. Medications taken during pregnancy were Bendectin (pyridoxine hydrochloride and doxylamine succinate), Chlor-Trimeton (chlorpheniramine maleate), and Sudafed (pseudoephedrine), and prenatal vitamins. The total gain in weight during the pregnancy was 34 pounds. A dramatic increase in fundal height 2 weeks before delivery prompted a real-time ultrasound examination, which showed massive polyhydramnios and a fetal head size of 30 weeks with no apparent fetal anomalies. Amniocentesis showed a lecithin/sphingomyelin ratio of 8.0. Onset of labor was spontaneous. Cesarean section

was performed for fetal distress. There was a tight nuchal cord  $\times 6$ . Apgar scores were 0 at 1 and 5 minutes, and resuscitative efforts were unsuccessful.

Family history was unremarkable, except for a maternal uncle who had died at 2 days of age from an unknown heart problem.

Physical examination disclosed that the premature infant was small for gestational age and grossly normal in appearance. All size parameters were far below the tenth percentile for a 35-week fetus and appropriate for a 30-week fetus. The head and neck showed normal morphologic features. The chest was asymmetric with left-sided prominence. Three umbilical vessels were identified. The labia majora were moderately hypoplastic. The anus was patent. Brachyclinodactyly of the fifth fingers, with absence of the distal interphalangeal crease, was present bilaterally. The limbs were otherwise normal, and the fingers were not overlapping.

Radiologic examination revealed 11 pairs of ribs, with the ribs on the left side longer than those on the right. The hands were symmetric, with hypoplastic second middle phalanges, and hypoplastic fifth middle and distal phalanges with marked clinodactyly. The skeleton was otherwise normal, although the pelvis could not be evaluated from available films.

Postmortem examination revealed the following remarkable features. The left side of the chest contained intestines, stomach, spleen, and pancreas as a result of a large left diaphragmatic hernia, with the left hemidiaphragm represented by an anterior muscular band. The left side of the heart was severely hypoplastic, with only a small pouch representing the left atrium. A patent foramen ovale, ventricular septal defect, and bicuspid aortic valve were present, and coronary vessels were aberrant in course. The left lung was severely hypoplastic. The vagina was prolapsed. The placenta was small (250 grams), with a small wedge-shaped infarct.

Cytogenetic studies, including R banding, revealed a 47,XX+18 karyotype in each of eight metaphases from cultured lymphocytes. Cultured skin fibroblast karyotyping confirmed this finding in all 25 metaphases counted.

Remarkably, this small-for-gestational-age female infant with trisomy 18 lacked major external dysmorphic features and had only a single clinically apparent major malformation, diaphragmatic hernia. She showed none of the more typical dysmorphic features which generally lead to clinical suspicion of trisomy 18. Although the possibility of a chromosomal syndrome was not considered until complex cardiac malformations were found at postmortem examination, tissues for karyotyping were obtained as part of a routine protocol for stillbirths, and the diagnosis of trisomy 18 was made. As a result, the parents could be informed of a low (less than 1%) recurrence risk for chromosomal aneuploidy<sup>1</sup> and the availability of prenatal chromosomal analysis.

A standardized protocol for the evaluation of every stillborn infant should include photographs, X-ray films, complete autopsy, and banded karyotyping. Blood and another tissue, such as skin or gonad, should be obtained as soon as possible after delivery to assure an adequate number of cultured cells for banded chromosomal analysis. Skin or gonad cells can be held in tissue culture medium pending the results of postmortem examination and may be discarded if

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multiple malformations are not found, thus maximizing cost effectiveness.

Chromosomal abnormalities are generally and appropriately considered in any infant with multisystem anomalies. In this case in which, initially, only a single malformation was thought to exist, tissues for chromosomal studies might not have been obtained if there had not been a standard protocol for the evaluation of stillbirths. Since the overall incidence of chromosomal anomalies in stillborn infants is 5% to 7%,<sup>2</sup> the yield of this protocol is sufficiently high to warrant routine evaluation. It allows for both the diagnosis of chromosomal abnormalities and the possibility of preventing recurrence by subsequent prenatal chromosomal testing.

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### Uterus didelphys unicollis with adenocarcinoma in one horn and atypical endometrial hyperplasia in the other: Case report

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THE HISTORY of the patient who is the subject of this report exemplifies the difficulties involved in making the correct diagnosis. Braun<sup>1</sup> reviewed the English literature and reported eight patients in whom the delay in diagnosis ranged from 3 months to 3 years, since in no patient were both cavities curetted at the original procedure. Other reports\* also emphasize this problem.

M. Y., an unmarried, nulligravid, white woman, 57 years old, was first seen in May, 1978, complaining of urinary frequency with incontinence. Menarche had occurred at age 11. The menstrual cycles had always been regular but were associated with severe cramps and prolonged bleeding. There had been no bleeding since an abrupt and spontaneous menopause 8 years earlier. Prior Papanicolaou smears had all been negative. The patient denied having had any operations or

major illnesses, except for hypertension that had been treated with diuretics. Examination disclosed that the patient was obese and hypertensive but in no acute distress. The uterus was small and irregular. The cervix was clean. There was a slight, but symptomatic, cystourethrocele. Vaginal cytologic findings showed a very good estrogenic effect. She was taught Kegel's exercises, and a Reisser pessary was inserted for support. Papanicolaou smears were reported to be negative. The pessary gave excellent support and was cleansed and repositioned at intervals.

One year later, she developed a profuse, watery discharge, which later became tinged with blood. Comprehensive investigation failed to identify a urinary tract fistula or vaginal ulcer. Vaginal cytologic findings were still highly estrogenic, and there was no change in the size of the uterus. At endometrial biopsy, the uterus was found to be 3½ inches deep, with an apparent stenosis or atresia at the internal os. Examination was not completely satisfactory, but no submucous fibroids were felt. The biopsy specimen (Fig. 1, A) was reported as showing involutinal endometrial and endocervical fragments without evidence of atypia. Because a pinkish discharge continued, curettage with the use of anesthesia was performed, and a moderately well-differentiated papillary adenocarcinoma of the endometrium was reported (Fig. 1, B).

The patient was hospitalized 1 week later for panhysterectomy. Preoperative evaluation disclosed that the patient was obese and hypertensive and had a severely hypoplastic left kidney. The remainder of the findings were within normal limits, except for an abnormal electrocardiogram. The patient withstood the operative procedure very well and convalesced without difficulty. The correct diagnosis was made only after the pathologist (K. A. S.) examined the specimen: The findings were uterus didelphys unicollis with invasive endometrial carcinoma of the left horn (Fig. 2, A) and atypical adenomatous hyperplasia of the endometrium in the right horn (Fig. 2, B). The two fundi represented what had been called fibroids. Tubes, ovaries, and vaginal cuff were free of malignant infiltration. Because of the extent of myometrial invasion, the patient received two applications of colpostat radiation by Nolan applicators, totaling 6,906 rads to the vaginal apex. Convalescence has continued satisfactorily, and there are no urinary problems.

Nicholson and associates<sup>2</sup> were the first to classify and subdivide the malignant malformed uteri into anatomic divisions. Because of duplication and incompleteness in some reports, there is difficulty in giving the exact number of cases reported, but it probably exceeds 55. In reviewing the available reports,\* we were unable to identify any patient with simultaneous primary tumors in both horns, although this has been reported in cervical malignancy. Tsibris and associates<sup>3</sup> studied estrogen and progesterone receptors in a bicornuate uterus and reported that each horn had normal distribution of both receptors.

Our patient had adenocarcinoma in one horn, along with atypical endometrial hyperplasia in the other, which suggests that each horn responded to the initiating factors. It is possible that, had we delayed longer, this patient might have been the first with a primary adenocarcinoma in each horn. We did not study estrogen or progesterone receptors in this uterus. Other

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\*Full bibliography available on request.



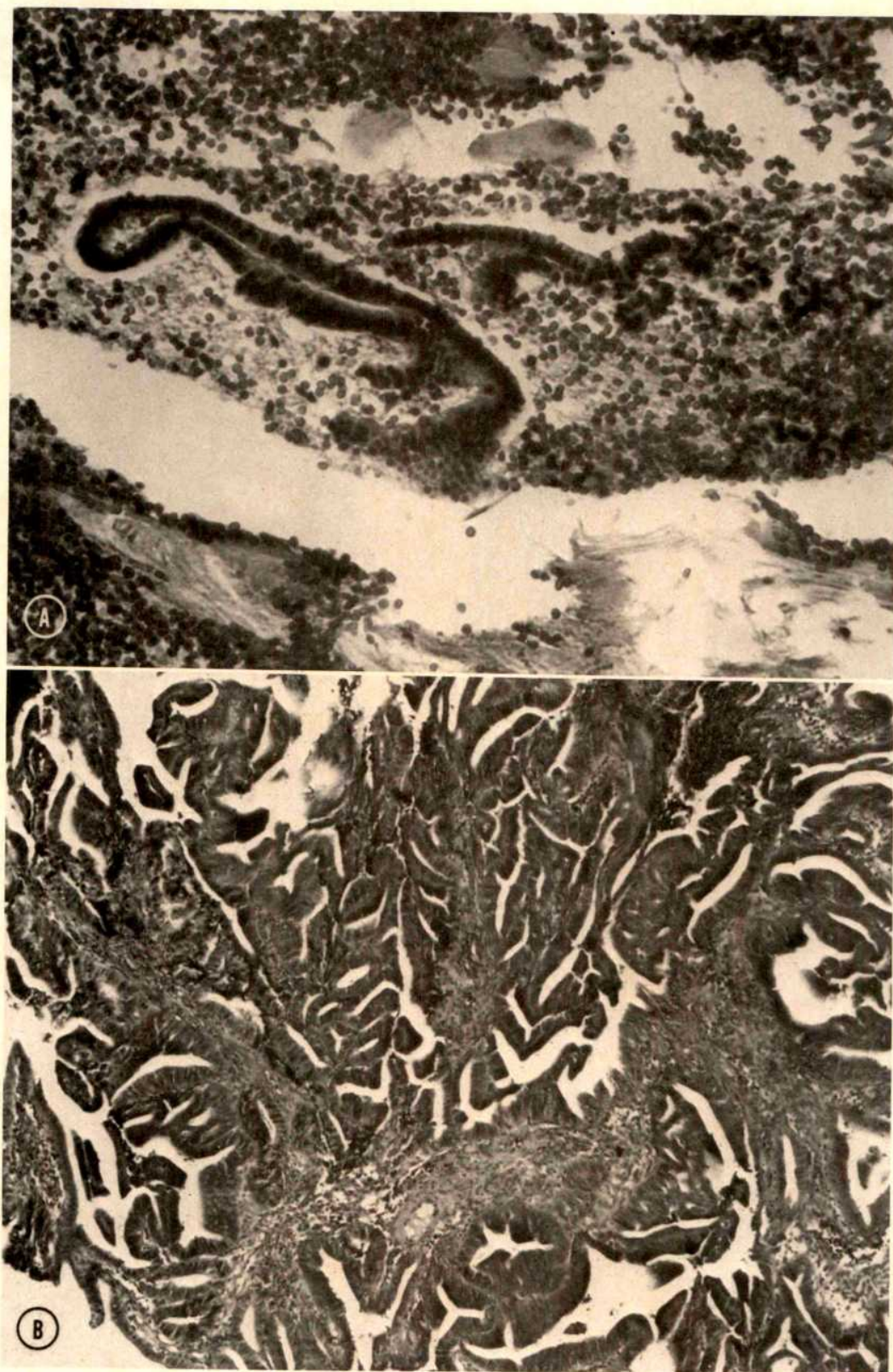


**Fig. 1.** Prehysterectomy specimens. *A*, Endometrial biopsy specimen (79-S-5893) showing red cells and fragments of endometrial epithelium without atypia. *B*, Curettage specimen (79-S-7585) showing moderately well-differentiated adenocarcinoma of the endometrium, in part papillary.



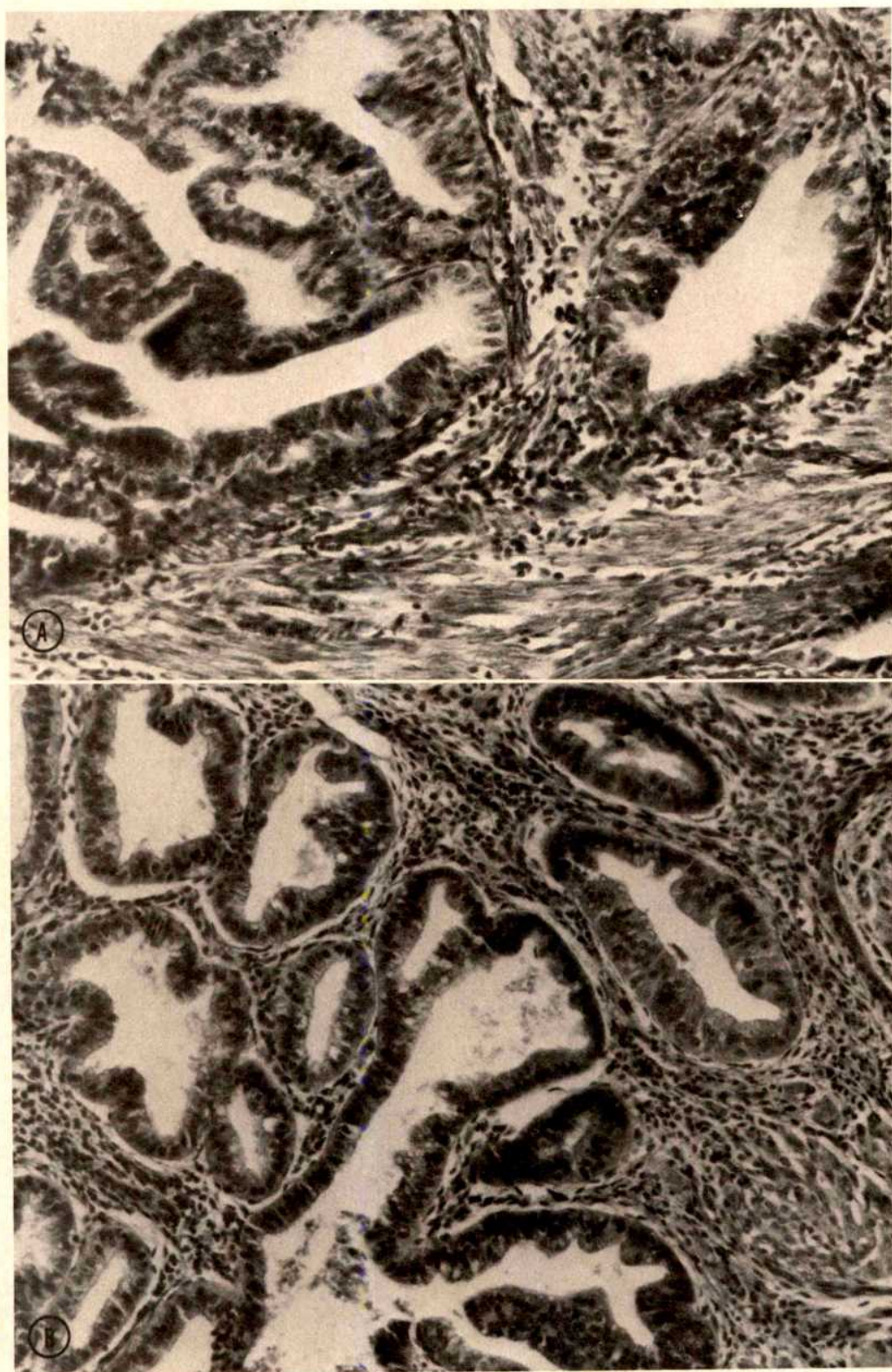


**Fig. 2.** Hysterectomy specimens. *A*, Right horn (79-S-7737). Adenocarcinoma in papillary and cribriform pattern, invading the myometrium. *B*, Left horn (79-S-7737). Endometrium with irregular proliferation of the glands, papillary infolding, and a suggestion of back-to-back pattern. Nuclei are variably placed, and some are large and hyperchromatic. A rare mitosis is seen. These changes are focally distributed in an otherwise menopausal endometrium.



reports\* recorded adenocarcinoma ranging from well differentiated to completely anaplastic. Other malignancies recorded were choriocarcinoma and carcinosarcoma.

We wish to thank Ms. L. Landau and the hospital librarians for their exhaustive search of the literature, and Mr. R. Newhouse and his staff for the microphotographs.

\*Full bibliography available on request.

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## CORRESPONDENCE

### Gonadotropin binding sites in ovaries of patients with familial XY gonadal dysgenesis and Turner's syndrome

To the Editors:

Patients with the XY gonadal dysgenesis syndrome develop fibrous streaks of ovarian tissue that have little or no normal ovarian morphology or function. The above is also true in patients with Turner's syndrome, but, in addition, in some instances their ovaries may contain scarce but some normal ovarian tissue elements. In both these syndromes, particularly in the former, one may expect that the ovarian tissue will not contain any gonadotropin binding sites because the tissue elements (i.e., corpus luteum and follicles) that are traditionally considered to possess gonadotropin binding sites are either absent or scarce.

Two ovarian specimens from two sisters with familial XY gonadal dysgenesis and one from a patient with Turner's syndrome (46, X ring X karyotype on chromosome analysis) were collected when the patients underwent surgery in the University of Louisville Hospital. The case histories, chromosome analysis, ovarian histology, and other relevant information on patients with XY gonadal dysgenesis can be found in another article (submitted for publication). Similar information on patients with Turner's syndrome is yet to be published. The ovarian tissues were homogenized and specific binding of  $^{125}\text{I}$ -human chorionic gonadotropin ( $^{125}\text{I}$ -hCG) to homogenates was determined.

The specific binding of  $^{125}\text{I}$ -hCG (fmol/mg of protein) in the ovarian homogenate of one sister was  $5.3 \pm 1.3$  (SEM); in the other sister it was  $2.5 \pm 0.9$ , and in the Turner's syndrome patient it was  $3.5 \pm 0.9$ . Thus, detecting specific binding when we did not expect to observe any to begin with and, more important, in the range we observed for some normal human corpora lutea of the menstrual cycle and term pregnancy came as a surprise. The immediate question in our mind was what ovarian tissue elements are responsible for the observed  $^{125}\text{I}$ -hCG binding? The ovaries of the XY gonadal dysgenesis patients contained stromal, hilar cells and remnants of rete ovarii. There is previous evidence that the former two cell types in human postmenopausal ovaries contain binding sites for luteinizing and follicle-stimulating hormones (LH and FSH) and exhibit signs of steroidogenesis.<sup>1</sup> Therefore, it is a good possibility that stromal and hilar cells are responsible for  $^{125}\text{I}$ -hCG specific binding observed in ovaries of XY gonadal dysgenesis patients. The ovarian histology of the Turner's syndrome patient revealed

the presence of stromal, hilar, granulosa, and theca interna cells and an oocyte. The first four cell types are possible contributors to observed  $^{125}\text{I}$ -hCG specific binding.

All three of these patients showed signs of exposure to sex steroids (secondary sexual characteristics). Although the origin of these steroids is unknown, the present results and previous data allow us to speculate that these steroids are, at least in part, of ovarian origin and probably under gonadotropin control (FSH and LH levels are elevated in XY gonadal dysgenesis patients and normal in the Turner's syndrome patient).

It is very difficult to obtain many ovarian tissue specimens from patients with XY gonadal dysgenesis and Turner's syndrome, for gonadotropin binding studies. Thus, the present finding in three different ovarian specimens should only serve to point out that the common expectation, which is that ovarian tissues from patients with XY gonadal dysgenesis and Turner's syndrome, do not contain gonadotropin binding sites, is untenable. In support of this are two important previous observations that ovaries which have little or no normal ovarian morphology or function (postmenopausal ovaries and premature ovarian failure) do contain gonadotropin binding sites.<sup>1, 2</sup>

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### Sinusoidal fetal heart rate pattern

To the Editors:

We read with interest the article by Young, Katz, and Wilson, entitled "Sinusoidal fetal heart rate. I. Clinical



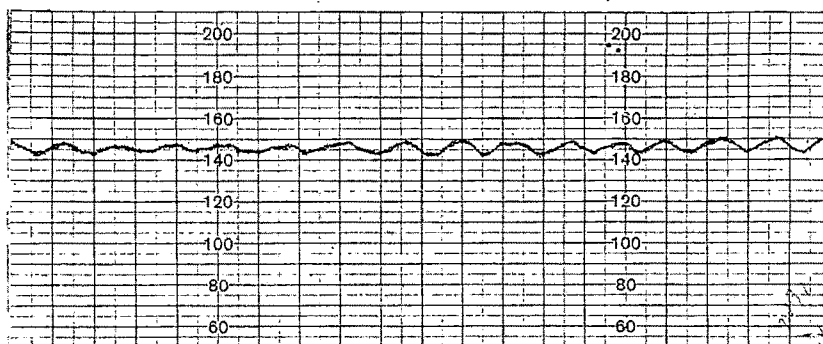


**Fig. 1.** Sinusoidal heart rate pattern from a severely affected baby of an Rh-sensitized mother. The baby was mildly hydropic with a hemoglobin at birth of 4 gm/100 ml. Note the uniform sinusoidal oscillations above and below the baseline and the absence of short-term variability.

significance" (*AM. J. OBSTET. GYNECOL.* 136:587, 1980). It is important to look at outcome in fetuses and newborn infants from a given population with an abnormality such as the sinusoidal heart rate pattern, especially when current knowledge is based on case reports. Furthermore, by looking at the overall population it represents, such information becomes valuable in terms of trying to appreciate the relative incidence.

However, we question whether or not the authors were indeed looking strictly at sinusoidal fetal heart rate patterns. The authors point out in their introduction that the sinusoidal fetal heart rate pattern is a regular rhythmicity of variation resembling a sinusoidal wave with a relatively fixed periodicity and amplitude and they also note that an absence of short-term variability has been associated with this pattern. However, the two fetal monitoring record examples (Figs. 3 and 4) which the authors present do not fit the usual criteria for sinusoidal patterns. In Fig. 4 there is a series of large amplitude accelerations above an identifiable baseline and short-term variability is present. This pattern, which they interpret as sinusoidal, seems to be more likely frequent accelerations of the fetal heart rate and return to baseline. We have noted that in true sinusoidal patterns (Fig. 1) the sine wave tends to fluctuate above and below a baseline and the more frequent "pseudosinusoidal" pattern can be distinguished by having normal short-term variability and oscillations fluctuating generally above the baseline. Such distinctions can be used to distinguish clearly the true, probably ominous sinusoidal pattern from the more frequently occurring pattern which probably represents only a uniformly increased long-term variability. In our experience the sinusoidal patterns that we have seen have been uniformly associated with poor outcome, with the use of much more strict criteria for designating the sinusoidal patterns.<sup>1, 2</sup> One exception to this has been the sinusoidal pattern which occurs with alphaprodine (Nisentil); however, this pattern is limited in timing, occurring for a relatively short time following the injection of this medication, and is seen in patients

in whom the heart rate patterns were previously and are subsequently normal and outcome is generally good.

We have been under the impression that there is some concern regarding the definition of the sinusoidal heart rate pattern and this article is an example of the confusion. This brings up two distinct problems: First, the practitioner looking at a fetal heart rate similar to the ones presented by the authors may intervene unnecessarily; second, a truly ominous sinusoidal fetal heart rate pattern with fetal jeopardy may be missed completely. In our experience true sinusoidal patterns which we have observed have been uniformly associated with poor fetal condition. This article does, however, point out that when there is any question whether or not one is dealing with a sinusoidal pattern or the more common "pseudosinusoidal pattern," one does need to perform further evaluations such as a fetal scalp pH determination to be certain.

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#### Reply to Drs. Garite, Modanlou, and Freeman

To the Editors:

Drs. Garite, Modanlou, and Freeman suggest that Figs. 3 and 4 in our paper do not "fit the usual criteria

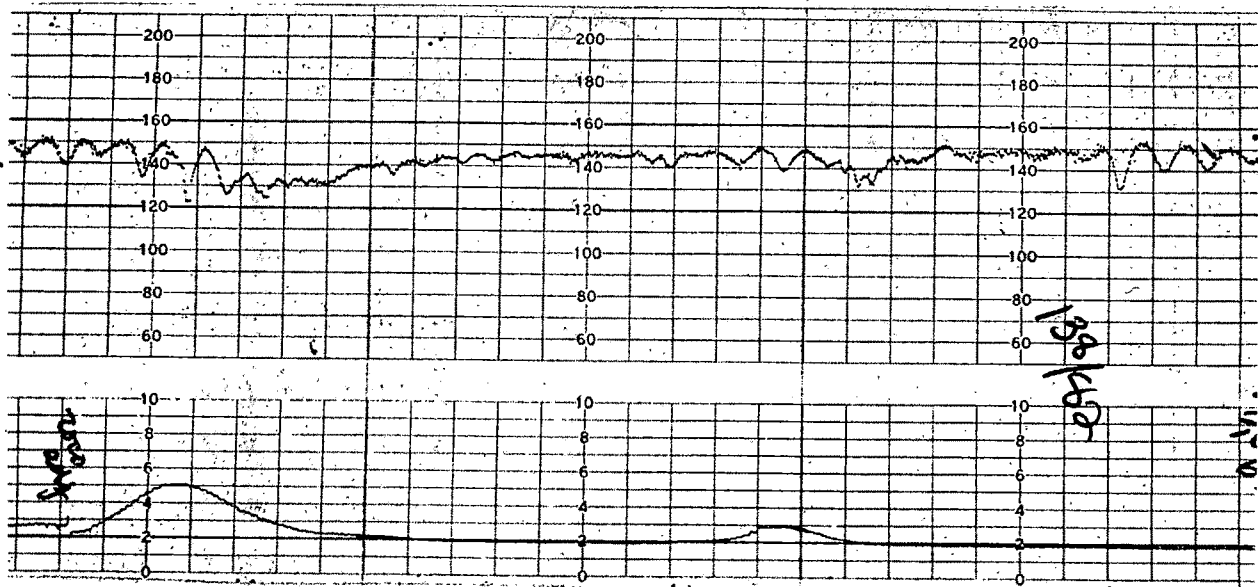


Fig. 1A. Portion of a tracing taken from Fig. 1, panel B. (From Modanlou, H., Freeman, R., Ortiz, O., et al.: Sinusoidal fetal heart rate pattern (FHR) and severe fetal anemia, *Obstet. Gynecol.* 49:537, 1977, published by The Williams & Wilkins Company.)

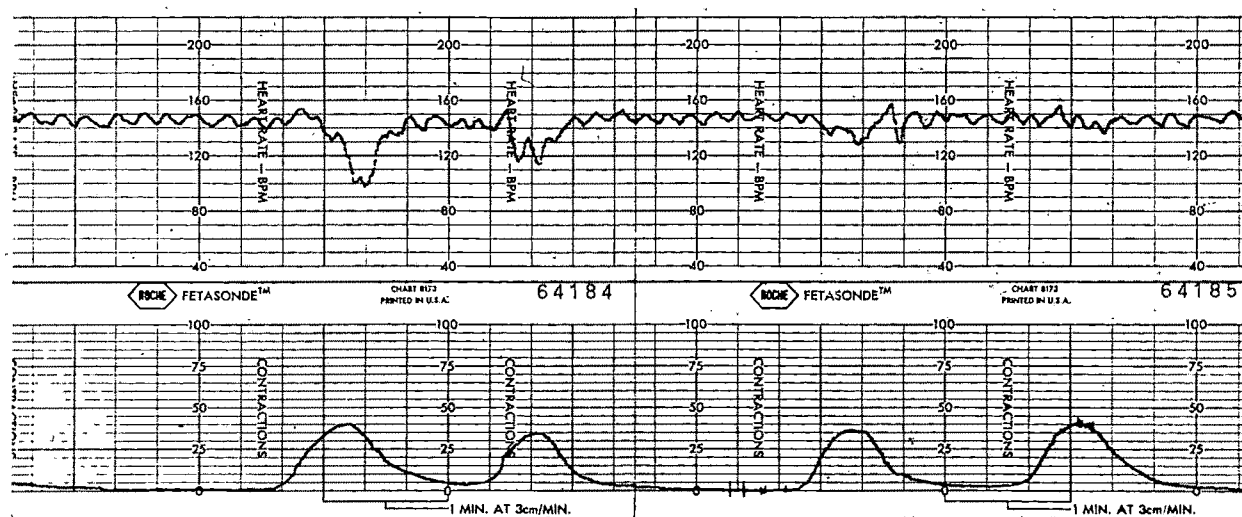


Fig. 1B. Portion of a tracing taken from Fig. 3. (From Young, B. K., Katz, M., and Wilson, S. J.: Sinusoidal fetal heart rate. I. Clinical significance, *AM. J. OBSTET. GYNECOL.* 136:587, 1980, published by The C. V. Mosby Company.)

for sinusoidal patterns." Fig. 3 illustrates a minor sinusoidal pattern of great interest because it is associated with variable decelerations. In the article<sup>1</sup> cited by Garite and associates, there is a very similar figure illustrating a sinusoidal heart rate associated with late decelerations (Figs. 1A and 1B).

They go on to define Fig. 4 as a "pseudosinusoidal" fetal heart rate, a new term. While this is an interesting notion, its use requires support by data. The writers disagree with our interpretation on the basis of the presence of short-term variability and their feeling that

there is fluctuation above an imagined baseline rather than around one. Neither consideration is part of the usual definition of a sinusoidal heart rate, on which our work was based.<sup>2-4</sup> The definition including the key determinants of a sinusoidal heart rate which we used was: a fetal heart rate pattern of regular variability resembling a sine wave, with a relatively fixed periodicity of 3 to 5 cpm and an amplitude of 5 to 40 bpm, present for at least 10 minutes. This is the strictest criterion published. Again, if we compare our Fig. 4 to a portion of the record in the case report<sup>1</sup> cited by Garite and



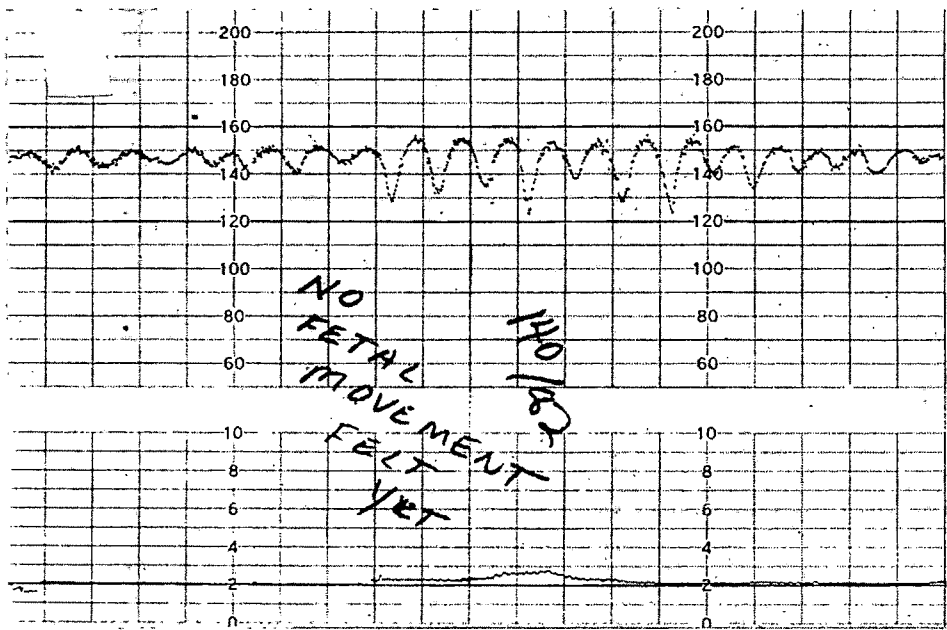


Fig. 2A. Portion of a tracing taken from Fig. 1, panel A. (From Modanlou, H., Freeman, R., Ortiz, O., et al.: Sinusoidal fetal heart rate pattern (FHR) and severe fetal anemia, *Obstet. Gynecol.* 49:537, 1977, published by The Williams & Wilkins Company.)

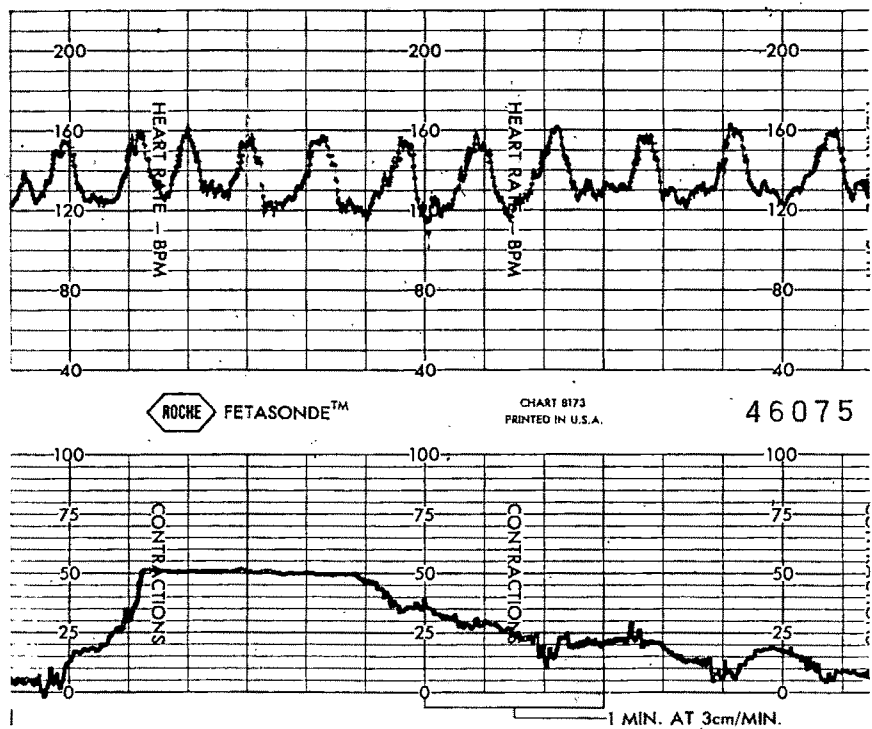


Fig. 2B. Portion of a tracing taken from Fig. 4. (From Young, B. K., Katz, M., and Wilson, S. J.: Sinusoidal fetal heart rate. I. Clinical significance, *AM. J. OBSTET. GYNECOL.* 136:587, 1980, published by The C. V. Mosby Company.)

associates, there is definite similarity in both sinusoidal heart rate illustrations (Figs. 2A and 2B).

Garite and associates also feel that our results are counter to their own experience; therefore, we must have been looking at "pseudosinusoidal" fetal heart rate patterns. They give two references to support their contention and define the "pseudosinusoidal" pattern. In the first article,<sup>1</sup> one case was reported and there was no mention of the "pseudosinusoidal" pattern. The sinusoidal heart rate in that case was associated with late deceleration in a positive stress test, indicating a strong likelihood of poor outcome even in the absence of a sinusoidal heart rate. The second reference they cite is unpublished. We look forward to seeing data in support of Dr. Garite's contention that there is a distinguishable "pseudosinusoidal" pattern with a good prognosis and a "true" sinusoidal pattern with an "ominous" prognosis. Our data did not reveal this, despite blood and tissue pH studies and analysis of various sinusoidal heart rate features for prognostic significance.

However, we must comment on the illustration provided by Garite and associates, presumably from their pending paper. No contraction channel is seen, and the legend indicates that this is "from a severely affected baby," making it unclear if this is a fetal or a neonatal record. In either case, the oscillation is from approximately 145 to 150 bpm. This is an amplitude of 2.5, thus below the minimum of 5 bpm required by the usual definition of a fetal sinusoidal heart rate.<sup>2-5</sup>

We agree with Garite and associates that there has been a problem of definition of the sinusoidal heart rate. Our publications attempted to resolve the problem by specifying a clear definition, verifying its applicability, and offering criteria for predicting fetal jeopardy based on biochemical data and outcome. We disagree with the suggestion that the practitioner may intervene unnecessarily based on our work. We advised scalp pH studies in all cases and provided specific criteria for cases of sinusoidal heart rate patterns with increased fetal risk based on correlation of the amplitude with pH and Apgar score. Our patients had no perinatal deaths and there were three cesarean sections for fetal distress out of 16 cases, an incidence of only 18.8%. In fact, the approach of Garite and associates is most likely to yield unnecessary intervention. They assert that the sinusoidal heart rate is "uniformly associated with poor fetal condition," and that a scalp pH determination is not needed unless there is a question of whether or not the pattern is sinusoidal. Such assertions can only result in a maximal incidence of cesarean sections.

Nor do we agree that by our approach a "truly ominous" pattern will be missed, a *non sequitur* if we are overdiagnosing the sinusoidal heart rate as they claim. We specifically stated that there was a 31% incidence of fetal distress in our series and advocated biochemical

monitoring of cases with sinusoidal heart rate patterns because of the high incidence of fetal jeopardy.

We have published our efforts toward a clear and practical approach to the sinusoidal heart rate, substantiated by biochemical data and Apgar scores. We look forward to additional data from other investigators which may contribute to progress with this clinical problem.

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#### Sinusoidal fetal heart rate

To the Editors:

Bruce K. Young, M.D., Miriam Katz, M.D., and Stephen J. Wilson, M.D., presented two studies of sinusoidal fetal heart rate in the March 1, 1980, issue of the *JOURNAL*, on pages 587 and 594.

They reported a better neonatal outcome for this pattern than had previously been shown. The reason is apparent. The pattern presented in Fig. 4 of the first article is not a sinusoidal pattern in spite of its undulating appearance but is simply increased variability, a pattern I commonly see with uterine hypertonus. Granted, it is indicative of a healthy fetus "struggling to maintain homeostasis in a strong compensatory effort under conditions of hypoxia" and thus not associated with poor fetal outcome if the hypoxia stress (e.g., tetanic labor) is relieved or the fetus is delivered. My contention is that this is *not* the sinusoidal pattern addressed by the earlier authors. The original term was based on the sine wave, quite unlike the tracing presented by Drs. Young, Katz, and Wilson. The article thus serves to confuse the literature on this subject when further clarification is what is needed.

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## Reply to Dr. Souma

To the Editors:

- Dr. Souma disagrees with the interpretation of Fig. 4 in the first of the two studies presented by Drs. Katz, Wilson, and myself but made no comment on the other two illustrative figures. Therefore, I assume that we all agree that the other two are sinusoidal patterns. However, the pattern in Fig. 4 conforms to the definition we offered and is specifically based on the literature.<sup>1-3</sup> This definition is a pattern of regular variability resembling a sine wave, with a relatively fixed periodicity of 3 to 5 cycles per minute and an amplitude of 5 to 40 bpm from the baseline fetal heart rate, lasting at least 10 minutes. Dr. Souma recognizes the pattern of Fig. 4 as "increased variability," notes "its undulating appearance," and agrees with our interpretation of its clinical significance.

Dr. Souma feels that the pattern in Fig. 4 does not resemble a sine wave closely enough. We think it does. Agreement on two and a half of three tracings is pretty good, for doctors.

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## Simultaneous real-time ultrasound scanning and pelvic examination

To the Editors:

Dr. Platt and associates have to be acknowledged for adding a European dimension to the American method of ultrasound investigation: simultaneous real-time ultrasound scanning (RTUS) and pelvic examination ("Simultaneous real-time ultrasound scanning and pelvic examination in assessment of pelvic disease," *AM. J. OBSTET. GYNECOL.* 136:693, 1980).

Certain cautions need to be expressed in interpreting their methods, in order not to discourage attempts of readers to apply this diagnostic aid in the investigation of pelvic abnormalities. First, and most important, there is no reason to refrain from utilizing the full bladder as an "acoustic window" for RTUS while performing a pelvic examination. On the contrary, both the displacement of the intestines to the upper abdo-

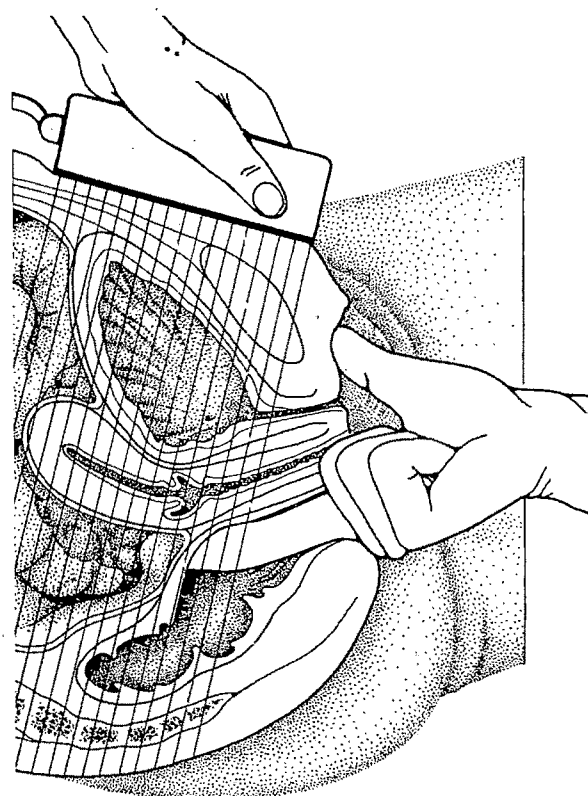


Fig. 1. Drawing demonstrating the use of RTUS simultaneously with pelvic examination.

men and the role of the bladder as a topographic marker and a tissue echo-density gauge facilitate delineating any disturbances in the dynamic interrelationship of the female internal organs. Second, the impression might be gathered from this article (Fig. 1) that American women do not have intestines, apart from the (empty!) rectum. In reality, however, both the mass of intestines in front of the uterus and the rectum with its contents behind the uterus hinder a correct outlining of the abnormality in question.

It has been the experience in our department, where simultaneous RTUS and pelvic examination were introduced in 1977, that rectal (and not vaginal) examination adds valuable information to the ultrasound pictures. This brings up our third remark: A finger in the rectum allows the investigator to reach behind the parametria, and abnormal findings in these regions, i.e., in the parametria, do not have to remain concealed to the eye of the gynecologist applying RTUS.

We suggest replacing Fig. 1 in the article by Platt and associates by our Fig. 1, illustrating our technique of combined pelvic examination, and hope that this may encourage even more gynecologists in the United States to reach for the RTUS transducer while doing a pelvic examination.

Art work was done by Ms. M. Ackermans-de Leeuw.

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Robert S. G. M. Bots, M.D.

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### Reply to Drs. Evers and Bots

To the Editors:

Drs. Evers and Bots raise three interesting points which warrant additional discussion. Their suggestion to utilize a full bladder for an acoustic window when performing the pelvic examination might be helpful in some situations. In our experience we have obtained more information with the bladder empty, thus allowing us to mobilize the uterus and approximate it to the anterior abdominal wall. Both techniques should be considered, depending on the clinical circumstances.

Their second point suggests that our drawing is inadequate. We would like to emphasize that this drawing is simply meant to demonstrate the combined technique, which it does. We do not believe that their drawing adds anything new.

Finally, their suggestion to use an examining finger in the rectum is well taken since we also believe this is part of a complete pelvic examination and can also be of benefit while scanning.

We wish to thank Drs. Evers and Bots for their interesting comments.

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### Effects of dietary lead and zinc

To the Editors:

While reading two recent papers by Dilts and Ahokas in the *AMERICAN JOURNAL OF OBSTETRICS AND GYNECOLOGY* on the effects of dietary lead and zinc on pregnancy<sup>1</sup> and fetal organ growth,<sup>2</sup> I detected several inappropriate statements regarding previously reported work by Cerklewski and Forbes<sup>3</sup> and Cerklewski.<sup>4</sup> With regard to reference 3, for example, which detailed an interaction between lead and zinc in young male rats, the authors stated on page 944 of the first publication that intestinal food absorption was affected in our study. No effect on food intake was observed in any of the nine treatments in this study or in the subsequent pregnancy study. Such an effect would have been difficult to overlook since biochemical toxicity of lead was reduced 50% in male rats. Second, the zinc and lead additions in my pregnancy study did not utilize

drinking water as the vehicle but rather the whole diet, which is contrary to the text of the authors. This difference itself may explain the divergent results obtained by the authors regarding an effect of lead on maternal food intake. In another example regarding the effects of zinc supplementation on growth-depressive effects of lead, the authors stated that "results are somewhat conflicting with those of Cerklewski." Comparison of the two papers makes it clear that this statement is inappropriate since it compares fetal weights at 21 days of gestation with pup weights at day 15 of lactation. Finally the discussants at the end of both papers by Dilts and Ahokas concluded that data are lacking concerning the effects of zinc supplementation on fetal lead toxicity. Dr. Dilts indicated in response that the data were forthcoming, with no reference to the fact that data have already been published in my abstract cited by Dilts and Ahokas and in a full publication.<sup>4</sup>

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### Reply to Dr. Cerklewski

To the Editors:

We wish to thank Dr. Cerklewski for pointing out an incorrect statement. In our December 1, 1979, publication, we stated that the principal action of lead in pregnant rats was impaired intestinal food absorption and cited Cerklewski's work with male rats.<sup>3</sup> This was indeed in error as Dr. Cerklewski observed no effect on food intake even in rats fed as much as 200 ppm of lead.

With regard to the divergence of our results, we agree that the mode of lead administration (i.e., in the drinking water) may explain the decrease in maternal food consumption. It does not explain, however, the observation that zinc (200 ppm) added to the drinking water of pregnant rats fed 200 ppm lead did not experience an improvement in maternal weight gain despite an increase in food consumption. Fetal growth was slightly improved, however, presumably because of the increased protein/energy intake, thus suggesting that fetal growth retardation is, at least at low levels of lead



consumption, a result of reduced maternal food consumption.

Our statement that our results are in conflict with Cerklewski's regarding the effects of zinc supplementation on the growth-depressive effects of lead is not inappropriate. The conflict we refer to is that fetal growth could not be significantly increased by zinc supplementation in our study,<sup>2</sup> while Dr. Cerklewski observed an almost complete reversal of growth retardation with zinc supplementation.<sup>4</sup>

Finally the data we refer to as forthcoming concern the effects of zinc supplementation on maternal and fetal accumulation of lead and essential trace elements. This work is not yet completed.

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Smoking, weight gain, and nutrition during pregnancy

To the Editors:

Meyer<sup>1, 2</sup> has subjected my work<sup>3, 4</sup> and that of Davies and associates<sup>5</sup> to detailed scrutiny and dismissed our observations that cigarette smoking can lead to decreased maternal weight gain during pregnancy. Therefore, it is more than a little ironic that the most recent data from the Ontario Study that she uses in this controversy strongly support a conclusion quite the opposite of hers, repeating past history in the Ontario Perinatal Mortality Study, which initially concluded, "There was no indication, either, of higher perinatal mortality among infants born to mothers who smoked."<sup>6</sup>

Comprehensive theories of the mechanisms for the toxicity of maternal cigarette smoking on the fetus must account for the markedly stronger effects with lower social status. For instance, when we scrutinized the data of the 1958 British Perinatal Mortality Survey, we found that the excess perinatal mortality with smoking in those of upper social status (classes I and II) was 12.9% but was over three times as high (44.0%) in classes IV and V.<sup>7</sup> Other smaller studies have consistently shown a differential effect of smoking by social status.

Interpretation of the data in Meyer's Table IIIA<sup>2</sup> appears straightforward: At term and with the assumption that the dichotomy of weight gain between those gaining more or less than 20 pounds is an undistorted index of the distribution of weight gains, smoking is unrelated to weight gain in private patients, heavy smoking is very strongly related in public patients, and light and moderate smoking is uncertainly related. (I suspect that the relationships would emerge more clearly if the data were age standardized, since lighter smokers are likely to be younger than control

Table I. Perinatal mortality related to smoking

|                  | Perinatal deaths/1,000  |      |      | Risk, relative to nonsmokers |      |
|------------------|-------------------------|------|------|------------------------------|------|
|                  | Packs of cigarettes/day |      |      | Packs of cigarettes/day      |      |
|                  | 0                       | <1   | 1+   | <1                           | 1+   |
| Private patients | 20.8                    | 25.6 | 27.1 | 1.23                         | 1.30 |
| Public patients  | 29.8                    | 35.8 | 52.1 | 1.20                         | 1.75 |

subjects and heavier smokers, older.) In any case, as will be clear from the following data, it is the outcome for the heavy smokers that requires further explanation.

In order to test the statistical significance of the relationship of cigarette smoking to weight gain among public patients, the numbers of subjects in each cell are needed. In the absence of response to my repeated requests to Meyer, I have had to estimate numbers of subjects and have assumed that 35% were delivered at 38 to 39 weeks' gestation and the remainder, at 40 weeks or later. If those smoking under one pack a day averaged eight cigarettes per day and those smoking a pack or more smoked, on the average, 24 cigarettes per day, for public patients the  $\chi^2$  for linear trend, according to the method described in Armitage's text, was 8.45 ( $p < 0.01$ ), and the total  $\chi^2$  was 8.46. The  $\chi^2$  for the departure from linear trend was 0.01. Thus, among public patients, there was a highly significant relationship of the proportion of women gaining less than 20 pounds at term with increasing amounts of smoking.

The data of Table I were taken from Fig. 8 of Meyer and associates,<sup>8</sup> based on the same Ontario study. Neither the devastating mortality among infants of public patients smoking one or more packs of cigarettes daily nor the relative sparing of infants of the private heavy smokers is accounted for by the patterns of either the distribution of low birth weight or placental complications (see Table II).<sup>9</sup>

Is it possible that the private patients smoking one or more packs a day may have protected their offspring by sustaining weight gain?

Our recent finding<sup>11</sup> in a poor, public clinic population that nutritional supplementation reversed the lowered birth weight associated with heavy smoking gives direct evidence that smoking can interact with nutrition in affecting the growth of the fetus. These results strengthen the possibility that nutrition may, in part, be mediating the differential effects of cigarette smoking across social class on both birth weight and perinatal mortality.

Thus, deficits in weight gain during pregnancy do occur with smoking, particularly with descending social status, parallel with effects on birth weight and perinatal mortality. Also, there is now direct evidence that

Table II. Distribution of low birth weight and placental complications

|                                           | Packs of cigarettes/day |      |      | Risk, relative to nonsmokers |      |
|-------------------------------------------|-------------------------|------|------|------------------------------|------|
|                                           |                         |      |      | Packs of cigarettes/day      |      |
|                                           | 0                       | <1   | 1+   | <1                           | 1+   |
| Birth weight <2,500 gm (%):               |                         |      |      |                              |      |
| Private patients                          | 4.4                     | 7.1  | 10.6 | 1.6                          | 2.4  |
| Public patients                           | 5.8                     | 10.3 | 16.5 | 1.8                          | 2.8  |
| Placental complications/1,000 births (%): |                         |      |      |                              |      |
| Private patients                          | 21.7                    | 27.2 | 41.1 | 1.25                         | 1.89 |
| Public patients                           | 25.2                    | 34.7 | 44.9 | 1.37                         | 1.78 |

cigarette smoking interacts with nutrition in affecting fetal growth.

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External versus internal fetal monitoring

To the Editors:

I find it to be very disturbing to learn only in the Discussion section of the article by Haesslein and Nis-

wander, entitled "Fetal distress in term pregnancies" (*AM. J. OBSTET. GYNECOL.* 137:245, 1980), that the authors made no distinction as to whether the electronic fetal monitoring (EFM) was either external (indirect) or internal (direct), when Dr. Haesslein specified for the first time that a monitor tracing was external in describing a misdiagnosed fetal heart tracing.

My own uncontrolled clinical experience and observations have led me to conclude that external monitoring, be it performed electronically or humanly, by auscultation, for term pregnant patients in labor, is equally beneficial, but that internal, or direct, electronic fetal monitoring is far superior to the aforementioned by virtue of its precision, sensitivity, and minimal influence by artifact. Many are the times that an external electronic fetal monitor tracing has been analyzed as being reassuring, only to have that reassurance rapidly, and disquietingly, reversed upon application of the direct internal fetal scalp electrode.

I find it difficult to believe that my personal experiences concerning the potential for sharp contrast and difference in diagnosis in external versus internal electronic fetal monitoring are isolated. Therefore, I would make a plea that in future studies reported there be some discriminating descriptions of electronic fetal monitoring segregating the external from the internal type, with full realization that many patients may have the benefit of both modalities during one labor. How many of the false positive and false negative tracings were obtained externally?

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Rigid blood glucose control in pregnant diabetic women

To the Editors:

As we have come to recognize the value of tight blood sugar control in insulin-dependent diabetic pa-



tients during pregnancy, we are reminded by Leveno and colleagues<sup>1</sup> that perhaps such rigid control is unnecessary for a successful perinatal outcome. In my opinion, this may be appealing, but upon more detailed observation and analysis, it may not be as cost effective as one would think. Liberal and prolonged hospitalizations in most cases may not be cost effective if one looks at the complications, some of which may be monitored and averted on an outpatient basis. This is especially true when one considers that intensive monitoring, such as estriol and the nonstress test, was not essential and was not part of their protocol. Indeed, one may attempt, in well-selected and highly motivated patients, tight control and close monitoring at minimal costs. In two studies, one from Texas<sup>1</sup> and one from Italy,<sup>2</sup> the authors achieved good results. These studies are similar in that they did not require highly sophisticated monitoring techniques. They are also similar in that little hospitalization initially (2 to 3 days) was needed and a more prolonged period (4 to 6 weeks) was required prior to delivery.

I wonder if hospitalization, which was liberal in both of these series, would be enough to achieve good results. In the series from Italy it was emphasized that the insulin requirements were well established and spontaneous labor was anticipated. The study from Texas emphasized that the authors expected to maintain pregnancy until 37 weeks, at which time the lecithin/sphingomyelin ratio would be determined. If the ratio indicated fetal maturity, delivery was accomplished. Delivery was also performed in the presence of complications such as pre-eclampsia, pyelonephritis, or diabetic deterioration.

Although Levino and associates<sup>1</sup> maintain that the degree of control did not correlate with the statistically different perinatal mortality, one can see, with careful analysis, that the maternal and neonatal complication rates were more closely associated with the degree of control. On one hand, they showed a maternal complication rate of 9/17 in Group I, 47/79 in Group II, and 37/24 in Group III with a length of gestation at delivery of  $36.8 \pm 0.4$ ,  $36.7 \pm 0.1$ , and  $35 \pm 0.4$  weeks for the respective groups. On the other hand, neonatal complications were 17/89 in Group I, 87/77 in Group II, and 28/21 in Group III.

Although in their conclusions they state, "We found no such (beneficial) effects from rigid glucose control" and glucose control should be practical and realistically achievable, I feel one should not lose sight of their high incidence of preterm deliveries. The vast majority of these were because of the development of complications. In addition, numerous neonatal complications may reflect preterm delivery, all of which may be related to the degree of control. Therefore, it seems logical then that optimal perinatal outcome would be directly related to the degree of diabetic control.

Thus, indirectly one may argue that tighter, more

rigid control will prevent many complications and this in turn may hopefully lead to an improved quality of babies. Perhaps one ought not to judge success by such a crude measurement as the mortality rate but rather by the quality of the survivors.

I invite comments from authors as well as from other concerned investigators regarding the trend in management of pregnant diabetic patients as it concerns the degree of blood sugar control and the intensity of fetal surveillance so that we may explore the limits and direction in which we are moving.

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#### Reply to Dr. Semchyshyn

*To the Editors:*

Dr. Semchyshyn correctly points out that extensive hospitalization was an important factor in achieving successful pregnancy for our patients. We must emphasize that our liberal use of hospitalization was in part for the purpose of improving glucose control according to the prevailing national trend in this direction. Unquestionably, "... in well-selected and highly motivated patients ..." ambulatory management can be successful. The patient population at Parkland Memorial Hospital is primarily indigent and we find that in-hospital management most dependably provides our patients with the care they need.

Dr. Semchyshyn suggests use of antepartum electronic and hormonal tests of fetal-placental condition as a replacement for prolonged hospitalization. He also suggests that these tests may be useful in preventing preterm delivery and the associated neonatal complications of prematurity. We agree that certain neonatal complications are the result of preterm delivery. It is impossible for us to address directly the role of stress tests and estriol measurements in preventing preterm delivery since we did not use these methods of fetal surveillance. In a report by Gabbe and associates,<sup>1</sup> antepartum testing permitted 35% of women to be delivered beyond 38 weeks of gestation. However, as

pointed out by Dr. Quilligan in the published discussion of our paper, their neonatal morbidity figures were not too different from those reported by us. Again, according to the report by Gabbe and associates, the mean gestational age when antepartum tests became abnormal was 37.4 weeks. Women whose tests remained normal had a mean gestational age at delivery of 38 weeks. Since antepartum tests became abnormal at almost the same time women with normal tests were delivered, it can be concluded that these tests contributed little to prolonging pregnancy beyond 37 or 38 weeks. A more recent report by Schneider and associates<sup>2</sup> describes use of the oxytocin challenge test as well as urinary estriol measurement during outpatient management. The patient population was "middle class rural." The perinatal mortality rate was 2.7%, which attests to the feasibility of outpatient management. However, only about 20% of their patients were delivered at or beyond 38 weeks and the overall neonatal complication rate was 75.2%. From these results, it appears that antepartum testing for the purpose of extending pregnancy beyond 38 weeks does not impact on large numbers of diabetic women and does not abolish neonatal morbidity. Schneider and associates found urinary estriol determinations of no value in the management of pregnant diabetic patients, in contrast to the usefulness of the oxytocin challenge test.

In Dr. Semchyshyn's reanalysis of our data regarding the relationship of glucose control to neonatal and maternal complications, he emphasized our conclusions. Poor control (mean plasma glucose level  $>172$  mg/dl) is to be avoided but rigid control (glucose level  $<115$  mg/dl) is not clearly superior to good control (glucose level 115 to 172 mg/dl). By summing up the number of complications in each glucose control group, Dr. Semchyshyn nicely reveals that pregnancy complicated by overt diabetes is truly high risk and frequently so in spite of euglycemia.

The twentieth century has been a remarkable time for the diabetic woman who became pregnant. The prospects for successful pregnancy improved from only 40% at the turn of the century to 85% by the 1960s.<sup>3</sup> This improvement has continued during the 1970s as 95% or more of diabetic women can now expect a successful pregnancy. Interestingly, it is only during the past decade that rigid control has evolved into a centerpiece in the management armamentarium. A major impetus in this evolution was provided by Karlsson and Kjellmer's<sup>4</sup> report in 1972. This report, which has served as a model for the presentation of our data, described statistically significant associations between rigid glucose control and reductions in perinatal mortality as well as neonatal morbidity. Somewhat unappreciated in this report was the fact that only 31% of women had rigid control of diabetes and thus could

avail themselves of the perinatal benefits. Since this report, most investigators have attested to the value of rigid glucose control, yet seldom have they achieved this degree of control in even a majority of their insulin-dependent patients. In an exceptional report by Coustan and associates,<sup>5</sup> an entire group of 73 insulin-dependent women had rigid control as defined by a mean plasma glucose level of 108 mg/dl. Ninety-six percent of these women had successful pregnancies but the incidence of maternal hypoglycemia was striking. Approximately 38% of their patients either needed help to ingest calories or were brought to an emergency room because of hypoglycemia. Although these women apparently suffered no serious consequences, the specter of such hypoglycemia in unattended patients is frightening. Despite the apparent inability of many investigators to control rigidly a majority of diabetic women during pregnancy and the hazard of maternal hypoglycemia, the quest continues, regardless of the fact that 95% of diabetic women have successful pregnancies and by no means are these 95% euglycemic.

The controversy regarding the benefits of euglycemia is not limited to the discipline of obstetrics. Our colleagues in internal medicine are also divided on the importance of euglycemia in preventing diabetic vascular complications.<sup>6, 7</sup> Moreover, they debate the definition of "rigid" glucose control. In this regard, one colleague stated, "... 'tight control' comes closer to being a philosophical concept than a phenomenon one can quantify."<sup>8</sup> Thus, while our zeal is well intentioned, we have not been extremely successful at defining rigid control, at agreeing on its benefits, or in delivering this degree of control to very many of our patients. It is time to appraise these realities.

We wish to thank Dr. Semchyshyn for his letter urging an exploration of the "... trend in management of pregnant diabetic patients as it concerns the degree of blood sugar control..." This was the purpose of our report.

Kenneth J. Leveno, M.D.

John C. Hauth, M.D.

Larry C. Gilstrap III, M.D.

Peggy J. Whalley, M.D.

Department of Obstetrics and Gynecology  
University of Texas Southwestern Medical School  
Dallas, Texas 75235

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pregnancies in relation to the mother's blood sugar level, *AM. J. OBSTET. GYNECOL.* **112**:213, 1972.

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### The Gibson incision

#### *To the Editors:*

The extraperitoneal surgical approach to the pelvis is a valuable technique that should be part of the armamentarium of the well-trained pelvic surgeon. When access to the major pelvic blood vessels is essential, whether it be for obstetric or gynecologic indications, such as postpartum hemorrhage or posthysterectomy hemorrhage from the vaginal cuff, when there is coexistent peritonitis, an extraperitoneal surgical approach to the pelvis may well be the best one with transperitoneal surgical approaches being contraindicated.

When I was taught the various incisions for entering the extraperitoneal pelvic area, one of the incisions was named the modified Gibson incision.<sup>1</sup> Dr. Helmut Schellhas, however, refers in his report, "Extraperitoneal surgical approach to the pelvis after radiation therapy or radical surgery" (*AM. J. OBSTET. GYNECOL.* **137**:367, 1980), to the "Gibbons incision." What's in a

name? The extraperitoneal pelvic area is exposed as well whether it be entered via the incision of Gibson or Gibbons. Nevertheless, clarity of communication is essential and I would be interested to know whether the printing of the eponym Gibbons was a typographic error that was actually meant to be printed as Gibson and, if not, what the differences are between the incision of Gibbons and that of Gibson.

Sylvain Fribourg, M.D., F.A.C.O.G.

*Department of Obstetrics and Gynecology  
Southern California Permanente Medical Group  
13652 Cantara Street  
Panorama City, California 91402*

### REFERENCE

1. Glenn, J. F.: *Urologic Surgery*, New York, 1969, Hoeber Medical Division, Harper & Row Publishers, Inc., pp. 151-153.

### Reply to Dr. Fribourg

#### *To the Editors:*

The correct eponym is "Gibson" for the pelvic extraperitoneal incision. I appreciate Dr. Fribourg's comment to this typographic error.

Helmut F. Schellhas, M.D., F.A.C.O.G.

*Department of Obstetrics and Gynecology  
University of Cincinnati Medical Center  
College of Medicine  
231 Bethesda Avenue  
Cincinnati, Ohio 45267*

# BOOKS

## Books received

- Eduard Pernkopf Atlas of Topographical and Applied Human Anatomy.** Vol. 2. Thorax, Abdomen and Extremities. Edited by Helmut Ferner. 418 pages, illustrated. Baltimore, Maryland, 1980, Urban & Schwarzenberg. \$98.00.
- Clinics In Andrology.** Vol. 1. **Homologous Artificial Insemination (AIH).** Edited by J. C. Emperaire, A. Audebert, and E. S. E. Hafez. 235 pages, illustrated. Hingham, Massachusetts, 1980, Kluwer Boston, Inc. \$47.35.
- Diagnostic Ultrasound Applied to Obstetrics and Gynecology.** Edited by Rudy E. Sabbagha. 434 pages, illustrated. Hagerstown, Maryland, 1980, Harper & Row Publishers, Inc. \$47.50.
- Functional Morphologic Changes in Female Sex Organs Induced by Exogenous Hormones.** International Symposium of the Section of Gynecopathology of the German Society of Pathology, Heidelberg, 1979. Edited by Gisela Dallenbach-Hellweg. 232 pages, illustrated. Secaucus, New Jersey, 1980, Springer-Verlag New York, Inc. \$32.00.
- Human Reproduction.** An Integrated View. J. I. D. Sadow, A. P. Gulamhusein, M. J. Morgan, N. J. Naftalin, and S. A. Petersen. 198 pages, illustrated. Chicago, 1980, Year Book Medical Publishers, Inc. \$14.95.
- Pediatric and Adolescent Endocrinology.** Vol. 7. **Nutrition and the Diabetic Child.** Volume Editors: Zvi Laron and Moshe Karp. Proceedings of the Fourth International Beilinson Symposium, Herzlia, May, 1978. 395 pages, illustrated. White Plains, New York, 1979, Albert J. Phiebig, Inc. \$119.50 (soft cover).
- Progress in Medical Ultrasound.** Reviews and Comments. Volume 1/1980. Edited by A. Kurjak. 335 pages, illustrated. New York, 1980, Elsevier/North Holland Inc. \$61.00.
- Therapeutic Progress in Ovarian Cancer, Testicular Cancer and the Sarcomas.** Edited by A. T. van Oosterom, F. M. Muggia, and F. J. Cleton. 507 pages, illustrated. Hingham, Massachusetts, 1980, Kluwer Boston, Inc. \$65.80.
- Urine Sediment Examination.** G. Berry Schumann. 213 pages, illustrated. Baltimore, Maryland, 1980, The Williams & Wilkins Co. No price listed.



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# INDEX TO ADVERTISERS

|                                                                                  |              |                                                                                                   |                        |
|----------------------------------------------------------------------------------|--------------|---------------------------------------------------------------------------------------------------|------------------------|
| Baystate Medical Center<br><i>Opportunity Available</i> -----                    | 43           | New York Hospital-Cornell Medical Center<br><i>Opportunity Available</i> -----                    | 22                     |
| Bren Laboratories Inc.<br><i>Fergon</i> -----                                    | 24           | Northwest Permanente, P.C.<br><i>Opportunity Available</i> -----                                  | 43                     |
| Capital Area Community Health Plan<br><i>Opportunity Available</i> -----         | 22           | Norwich-Eaton Pharmaceuticals<br><i>Macrochantin</i> -----                                        | 8                      |
| Duke University Medical Center<br><i>Opportunities Available</i> -----           | 17           | Ortho Pharmaceutical Corporation<br><i>Ortho-Novum 1/35</i> -----                                 | 12, 13, 14             |
| Hall Medical Publishers, G.K.<br><i>Book</i> -----                               | 41           | Purdue Frederick Company, The<br><i>Betadine Vaginitis Regimen</i> -----                          | 16, 17                 |
| Holland-Rantos Co., Inc.<br><i>Koromex</i> -----                                 | 6            | Roche Laboratories, Division of Hoffmann-La Roche Inc.<br><i>Azo Gantanol</i> -----               | 38, 39, 40             |
| Ives Laboratories Inc.<br><i>Synalgos-DC</i> -----                               | 42           | Searle Laboratories<br><i>Cu7/Tatum-T</i> -----                                                   | Second Cover; 1        |
| Jobst Institute, Inc.<br><i>Venous Pressure Gradient Support Stockings</i> ----- | 23           | <i>Flagyl</i> -----                                                                               | 45, 46, Third Cover    |
| Johns Hopkins Hospital, The<br><i>Fellowship</i> -----                           | 45           | <i>Flagyl I.V.</i> -----                                                                          | 25, 26, 27, 28, 29, 30 |
| Kauai Medical Group<br><i>Opportunity Available</i> -----                        | 45           | Southern Connecticut Community Health Plan<br><i>Opportunity Available</i> -----                  | 22                     |
| Mead Johnson Pharmaceutical Division<br><i>Peri-Colace</i> -----                 | Fourth Cover | Syntex<br><i>Anaprox</i> -----                                                                    | 31, 32, 33, 34, 35, 36 |
| Merck, Sharp & Dohme<br><i>Mefoxin</i> -----                                     | 2, 3, 4      | <i>Norinyl 1 + 50</i> -----                                                                       | 19, 20                 |
|                                                                                  |              | University Associates for International Health Incorporated<br><i>Opportunity Available</i> ----- | 43                     |
|                                                                                  |              | University of Mississippi Medical Center, The<br><i>Opportunities Available</i> -----             | 43                     |
|                                                                                  |              | University of Vermont, The<br><i>Opportunity Available</i> -----                                  | 22                     |

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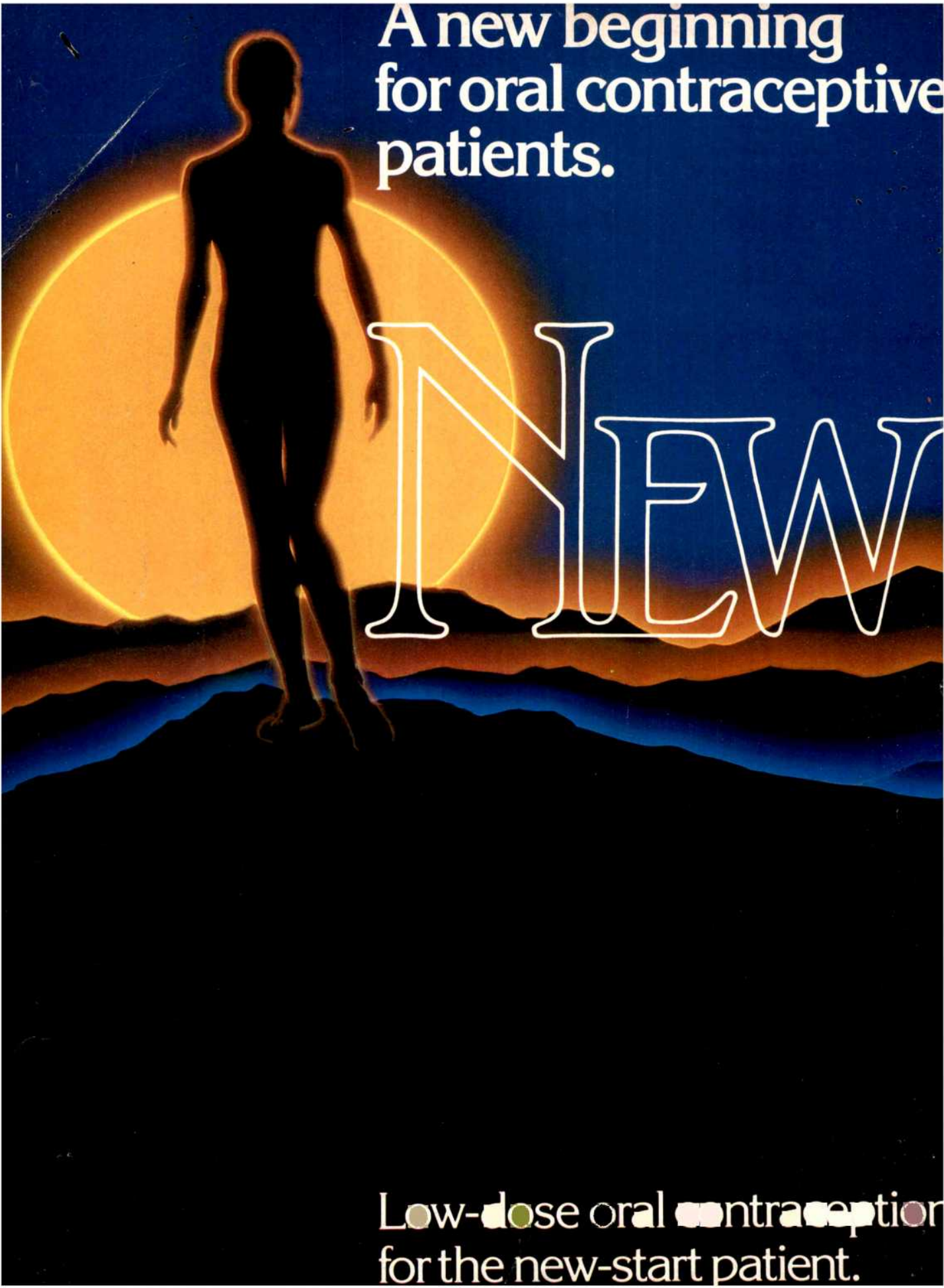


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**WARNINGS:** Cigarette smoking increases the risk of serious cardiovascular side effects from O.C. use. This risk increases with age and with heavy smoking (15 or more cigarettes per day) and is quite marked in women over 35 years of age. Women who use O.C.s should be strongly advised not to smoke.

The use of O.C.s is associated with increased risk of several serious conditions including thromboembolism, stroke, myocardial infarction, liver tumor, gall bladder disease, visual disturbances, fetal abnormalities, and hypertension. Practitioners prescribing O.C.s should be familiar with the following information relating to these risks.

1. **Thromboembolic Disorders and Other Vascular Problems:** An increased risk of thromboembolic and thrombotic disease associated with the use of O.C.s is well established. One British study demonstrated an increased relative risk for fatal venous thromboembolism; two British and three American studies demonstrated an increased relative risk for non-fatal venous thromboembolism. These studies estimate that users of O.C.s are 4 to 11 times more likely than nonusers to develop these diseases without evident cause. **CEREBROVASCULAR DISORDERS:** Two American studies demonstrated an increased relative risk for stroke, which had not been shown in prior British studies. In a collaborative American study of cerebrovascular disorders in women with and without predisposing causes, it was estimated that the relative risk of hemorrhagic stroke was 2.0 times greater in users than nonusers and the relative risk of thrombotic stroke was 4 to 5 times greater in users than in nonusers. A British long-term, follow-up study reported in 1976 a highly significant association between O.C. use and stroke. Another British long-term, follow-up study had suggested such an association in 1974, but the number of cases was too small to estimate the risk. **MYOCARDIAL INFARCTION:** An increased relative risk of myocardial infarction associated with the use of O.C.s has been reported, confirming a previously suspected association. One study conducted in the United Kingdom found, as expected, that the greater the number of underlying risk factors for coronary artery disease (cigarette smoking, hypertension, hypercholesterolemia, obesity, diabetes, history of preclampsia toxemia, and higher risk of developing myocardial infarction, regardless of whether the patient was an O.C. user or not, O.C.s, however, were found to be an additional risk factor. In terms of relative risk, it has been estimated that O.C. users who do not smoke (smoking is considered a major predisposing condition to myocardial infarction) are about twice as likely to have a fatal myocardial infarction as nonusers who do not smoke. O.C. users who are also smokers have about a 5-fold increased risk of fatal infarction compared to users who do not smoke, and about a 10- to 12-fold increased risk compared to nonusers who do not smoke. Furthermore, the number of cigarettes smoked also is an important factor. In determining the importance of these relative risks, however, the baseline rates for various age groups must be given serious consideration. (The estimates are based on British vital statistics which show acute myocardial infarction death rates 2- to 3-times less than in the U.S. for women in these age groups; consequently, actual U.S. death rates could be higher.) The importance of other predisposing conditions mentioned above in determining relative and absolute risks has not been quantified; other synergistic actions may exist. **RISK OF DOSE:** In an analysis of data derived from several national adverse reaction reporting systems, British investigators concluded that the risk of thromboembolism including coronary thrombosis is directly related to the dose of estrogen used in O.C.s. Preparations containing 0.1 mg or more of estrogen were associated with a higher risk of thromboembolism than those containing 0.05-0.08 mg of estrogen. Their analysis did suggest, however, that the quantity of estrogen may not be the sole factor involved. This finding has been supported by a study in the United States. Careful epidemiological studies to determine the degree of thromboembolic risk associated with progestogen-only O.C.s have not been performed. Cases of thromboembolic disease have been reported in women using these products, and they should not be presumed to be free of excess risk. **ESTIMATE OF EXCESS MORTALITY FROM CIRCULATORY DISEASE:** A large British prospective study estimated the mortality rate per 100,000 women per year from diseases of the circulatory system for users and nonusers of O.C.s according to age, smoking habits, and duration of use. The overall excess death rate annually from circulatory diseases for O.C. users was estimated to be 20 per 100,000 (ages 15-34—5/100,000; ages 35-44—33/100,000; ages 45-49—140/100,000). The risk is concentrated in older women, in those with a long duration of use, and in cigarette smokers, and may persist after discontinuation of O.C. use. It was not possible, however, to examine the interrelationships of age, smoking, and duration of use, nor to compare the effects of continuous vs. intermittent use. Although the study showed a 10-fold increase in death due to circulatory diseases in users for 5 or more years, all of these deaths occurred in women 35 or older. Until larger numbers of women under 35 with continuous use for 5 or more years are available, it is not possible to assess the magnitude of the relative risk for this younger age group. The available data from a variety of sources have been analyzed to estimate the risk of death associated with various methods of contraception. The estimates of risk of death for each method include the combined risk of the contraceptive method (e.g., thromboembolic and thrombotic disease in the case of O.C.s) plus the risk attributable to pregnancy or abortion in the event of method failure. This latter risk varies with the effectiveness of the contraceptive method. The findings of this analysis are shown in Table 1 below. The study concluded that the mortality associated with all methods of birth control is low and below that of childbearing, with the exception of O.C.s in women over 40 who smoke. (The rates given for pill only/smokers for each age group are for smokers as a class. For "heavy" smokers (more than 15 cigarettes a day), the rates given would be about double; for "light" smokers (less than 15 cigarettes a day), about 50 percent.) The lowest mortality is associated with the condom or diaphragm backed up by early abortion. The study also concluded that O.C. users who smoke, especially those over the age of 30, have a greater mortality risk than O.C. users who do not smoke.

**Table 1. The annual number of deaths associated with control of fertility and no control per 100,000 nonsterile women by regimen of control and age of women are:**

|                      | 15-19 | 20-24 | 25-29 | 30-34 | 35-39 | 40-44 |
|----------------------|-------|-------|-------|-------|-------|-------|
| No method            | 5.6   | 6.1   | 7.4   | 13.9  | 20.8  | 22.6  |
| Abortion only        | 1.2   | 1.6   | 1.8   | 1.7   | 1.9   | 1.2   |
| Pill only—nonsmokers | 1.3   | 1.4   | 1.4   | 2.2   | 4.5   | 7.1   |
| Pill only—smokers    | 1.5   | 1.6   | 1.6   | 10.8  | 13.4  | 58.9  |
| IUDs only            | 0.9   | 1.0   | 1.2   | 1.4   | 2.0   | 1.9   |
| Traditional          |       |       |       |       |       |       |
| Contraception only   | 1.1   | 1.6   | 2.0   | 3.6   | 5.0   | 4.2   |
| Traditional contra-  |       |       |       |       |       |       |
| ception and abortion | 0.2   | 0.2   | 0.3   | 0.3   | 0.3   | 0.2   |

The risk of thromboembolic and thrombotic disease associated with O.C.s increases with age after approximately age 30 and, for myocardial infarction, is further increased by hypertension, hyperlipidemias, obesity, diabetes, or history of preclampsia toxemia and especially by cigarette smoking. Based on

## AGE-RELATED RISK OF O.C.s

|                                 | Age | Below 30 | 30-39 | 40+  |
|---------------------------------|-----|----------|-------|------|
| Heavy smokers                   |     | C        | B     | A    |
| Light smokers                   |     | D        | C     | B    |
| Nonsmokers                      |     |          |       |      |
| (No predisposing conditions)    |     | D        | C, D  | C    |
| Nonsmokers                      |     |          |       |      |
| (other predisposing conditions) |     | C        | C, B  | B, A |

A—Use associated with very high risk. C—Use associated with moderate risk.  
B—Use associated with high risk. D—Use associated with low risk.

The physician and the patient should be alert to the earliest manifestations of thromboembolic and thrombotic disorders (e.g., thrombophlebitis, pulmonary embolism, cerebrovascular insufficiency, coronary occlusion, retinal thrombosis, and mesenteric thrombosis). Should any of these occur or be suspected, the drug should be discontinued immediately. A four- to six-fold increased risk of post-surgery thromboembolic complications has been reported in O.C. users. If feasible, O.C.s should be discontinued at least 4 weeks before surgery of a type associated with an increased risk of thromboembolism or prolonged immobilization. Data also suggest that the presence of varicose veins substantially increases the risk of superficial venous thrombosis of the leg, the risk depending on the severity of the varicosities. **Ocular Lesions:** There have been reports of neuro-ocular lesions such as optic neuritis or retinal thrombosis associated with the use of O.C.s. Discontinue O.C. medication if there is unexplained, sudden or gradual, partial or complete loss of vision; onset of proptosis or diplopia; papilledema, or retinal vascular lesions, and institute appropriate diagnostic and therapeutic measures. **Carcinoma:** Long-term continuous administration of either natural or synthetic estrogen in certain animal species increases the frequency of certain adenomas, benign or malignant, such as those of the breast, cervix, vagina, uterus, ovary, pituitary and liver. Certain synthetic progestogens, none currently contained in O.C.s, have been noted to increase the incidence of mammary nodules, benign and malignant, in dogs. Several retrospective case-control studies have reported an increased relative risk (3.1 to 13.9 times) associating endometrial carcinoma with the prolonged use of estrogens in postmenopausal women who took estrogen replacement medication to relieve menopause symptoms. One publication reported on the first 30 cases submitted by physicians to a registry of cases of adenocarcinoma of the endometrium in women under 40 on O.C.s. Of the adenocarcinomas found in women without predisposing risk factors for adenocarcinoma of the endometrium (e.g., irregular bleeding at the time O.C.s were first given, polycystic ovaries) nearly all occurred in women who had used a sequential O.C. These products are no longer marketed. No statistical association has been reported suggesting an increased risk of endometrial cancer in users of conventional combination or progestogen-only O.C.s, although individual cases have been reported. Several studies have shown no increased risk of breast cancer to women taking O.C.s or estrogens. In one study, however, while no overall increased risk of breast cancer was noted in women treated with O.C.s, a greater risk was suggested for the subgroups of O.C. users with documented benign breast disease and for long-term (2-4 years) users. One other study indicated an increasing risk of breast cancer in women taking menopause estrogens, which increased with duration of follow-up. A reduced occurrence of benign breast tumors in users of O.C.s has been well documented. In a prospective study of women with cervical dysplasia, there was an increase in severity and of conversion to cancer *in situ* in O.C. users compared with nonusers. This became statistically significant after 3 to 4 years of use. Nonreversal of dysplasia within the first 6 months of pill use was suggested to be predictive of progression after prolonged exposure. There have been other reports of microglandular hyperplasia of the cervix in users of O.C.s. In summary, there is at present no confirmed evidence from human studies of an increased risk of cancer associated with O.C.s. Close clinical surveillance of all women taking O.C.s is, nevertheless, essential. In all cases of undiagnosed persistent or recurrent abnormal vaginal bleeding, appropriate diagnostic measures should be taken to rule out malignancy. Women with a strong family history of breast cancer or who have breast nodules, fibrocystic disease or abnormal mammograms should be monitored with particular care if they elect to use O.C.s instead of other methods of contraception. **4. Liver Tumors:** Sudden severe abdominal pain or shock may be due to rupture and hemorrhage of a liver tumor. There have been reports associating benign or malignant liver tumors with O.C. use. This has been reported in short-term as well as long-term users of O.C.s. One study reported that use of O.C.s with high hormonal potency and age over 30 years may further increase a woman's risk of hepatocellular adenoma. Two studies relate with duration of use, the risk being much greater after 4 or more years of use. Although it is an uncommon lesion, it should be considered in women presenting with an "acute abdomen". The tumor may cause serious or fatal hemorrhage. Patients with liver tumors have demonstrated variable clinical features which may make preoperative diagnosis difficult. Some cases presented because of right upper quadrant masses, while most had signs and symptoms of acute intraperitoneal hemorrhage. Routine radiological and laboratory studies may not be helpful. Liver scans may clearly show a focal defect. Hepatic arteriography may be a useful procedure in diagnosing primary liver neoplasms. **5. Use in or Immediately Preceding Pregnancy, Birth Defects in Offspring, and Malignancy in Female Offspring:** The use of female sex hormones—both estrogenic and progestational agents—during early pregnancy may seriously damage the offspring. It has been shown that females exposed *in utero* to diethylstilbestrol, a nonsteroidal estrogen, have an increased risk of developing in later life a form of vaginal or cervical cancer that is ordinarily extremely rare. This risk has been estimated to be of the order of 1 in 1,000 exposures or less. Although there is no evidence at the present time that O.C.s further enhance the risk of developing this type of malignancy, such patients should be monitored with particular care if they elect to use O.C.s instead of other methods of contraception. Furthermore, a high percentage of women exposed to diethylstilbestrol (from 30 to 90%) have been found to have epithelial changes of the vagina and cervix. Although these changes are histologically benign, it is not known whether this condition is a precursor of vaginal malignancy. Male children so exposed may develop abnormalities of the urogenital tract. Although similar data are not available with the use of other estrogens, it cannot be presumed that they would not induce similar changes. An increased risk of congenital anomalies, including heart defects and limb defects, has been reported following use of sex hormones, including O.C.s, in pregnancy. In one case-control study it was estimated that there was a 4.7-fold increased relative risk of limb-reduction defects in infants exposed *in utero* to sex hormones (O.C.s, hormonal withdrawal tests for pregnancy or attempted treatment for threatened abortion). Some of these exposures were very short and involved only a few days of treatment. The data suggest that the risk of limb-reduction defects in exposed fetuses is somewhat less than 1 in 1,000 live births. In a large prospective study, cardiovascular defects in children born to women who received female hormones, including O.C.s, during early pregnancy occurred at a rate of 18.2 per 1,000 births, compared to 7.8 per 1,000 for children not so exposed *in utero*. These results are statistically significant. In the past, female sex hormones have been used during pregnancy in an attempt to treat threatened or habitual abortion. There is considerable evidence that estrogens are ineffective for these indications, and there is no evidence from well controlled studies that progestogens are effective for these uses. There is some evidence that triploidy and possibly other types of polyploidy are increased among abortifacients from women who become pregnant soon after ceasing O.C.s. Embryos with these anomalies are virtually always spontaneously aborted. Whether there is an overall increase in spontaneous abortion of pregnancies conceived soon after stopping O.C.s is unknown. If the patient has not adhered to the prescribed schedule, the possibility of pregnancy should be considered at the time of the first missed period (or after 45 days from the last menstrual period if progestogen-only O.C.s are used) and further use of O.C.s should be withheld until pregnancy has been ruled out. It is recommended that for any patient who has missed two consecutive periods, pregnancy should be ruled out before continuing the contraceptive regimen. If pregnancy is confirmed, the patient should be apprised of the potential hazard to the fetus and the advisability of continuation of the pregnancy should be discussed in the light of these risks. It is also recommended that women who discontinue O.C.s with the intent of becoming pregnant use an alternate form of contraception for a period of time before attempting to conceive. Many clinicians recommend 3 months, although no precise information is available on which to base this recommendation. The administration of progestogen-only or progestogen-estrogen combinations to induce withdrawal bleeding should not be used as a test of

another user an increased risk was apparent between 6 and 12 months of use. **7. Carbohydrate and Lipid Metabolic Effects:** A decrease in glucose tolerance has been observed in a significant percentage of patients on O.C.s. For this reason, prediabetic and diabetic patients should be carefully observed while receiving O.C.s. An increase in triglycerides and total phospholipids has been observed in patients receiving O.C.s. The clinical significance of this finding is unknown. **8. Elevated Blood Pressure:** An increase in blood pressure has been reported with O.C. use. In some women, hypertension may occur within a few months of beginning O.C. use. In the first year of use, the prevalence of women with hypertension is low in users and may be no higher than that of a comparable group of nonusers. The prevalence in users increases, however, with longer exposure, and in the fifth year of use is two and a half to three times the reported prevalence in the first year. Age is also strongly correlated with the development of hypertension in O.C. users. Women with a history of elevated blood pressure (hypertension), preexisting renal disease, a history of toxemia or elevated blood pressure during pregnancy, a familial tendency to hypertension or its consequences, or a history of excessive weight gain or fluid retention during the menstrual cycle may be more likely to develop elevation of blood pressure when given O.C.s and, therefore, should be monitored closely. Even though elevated blood pressure may remain within the "normal" range, the clinical implications of elevations should not be ignored and close surveillance is indicated, particularly for women with other risk factors for cardiovascular disease or stroke. High blood pressure may or may not persist after discontinuation of the O.C. **9. Headache:** The onset or exacerbation of migraine or development of headache of a new pattern which is recurrent, persistent, or severe, requires discontinuation of O.C.s and evaluation of the cause. **10. Bleeding Irregularities:** Breakthrough bleeding, spotting, and missed menses are frequent reasons for patients discontinuing O.C.s. In breakthrough bleeding, as in all cases of irregular bleeding from the vagina, nonfunctional causes should be borne in mind. In undiagnosed persistent or recurrent abnormal bleeding from the vagina, adequate diagnostic measures are indicated to rule out pregnancy or malignancy. If pathology has been excluded, time or a change to another formulation may solve the problem. Changing to an O.C. with a higher estrogen content, while potentially useful in minimizing menstrual irregularity, should be done only if necessary since this may increase the risk of thromboembolism. Women with a past history of oligomenorrhea or secondary amenorrhea or young women without regular cycles may have a tendency to remain anovulatory or to become amenorrheic after discontinuation of O.C.s. Women with these preexisting problems should be advised of this possibility and encouraged to use other contraceptive methods. Post-use anovulation, possibly prolonged, may also occur in women without previous irregularities. A higher incidence of galactorrhea and of pituitary tumors (e.g., adenomas) has been associated with amenorrhea in former users compared with nonusers. One study reported a 16-fold increased prevalence of pituitary prolactin-secreting tumors among patients with postpill amenorrhea when galactorrhea was present. **11. Infertility:** There is evidence of impairment of fertility in women discontinuing O.C.s in comparison with those discontinuing other methods of contraception. The impairment appears to be independent of the duration of use of the preparations. While the impairment diminishes with time, there is still an appreciable difference in the results in nulliparous women for the O.C. and non-O.C. groups 30 months after discontinuation of birth control. For parous women the difference is no longer apparent 30 months after cessation of contraception. **12. Ectopic Pregnancy:** Ectopic as well as intrauterine pregnancy may occur in contraceptive failures. However, in progestogen-only O.C. failures, the ratio of ectopic to intrauterine pregnancies is higher than in women who are not receiving O.C.s, since the drugs are more effective in preventing intrauterine than ectopic pregnancies. **13. Breast Feeding:** O.C.s given in the postpartum period may interfere with lactation. There may be a decrease in the quantity and quality of the breast milk. Furthermore, a small fraction of the hormonal agents in O.C.s has been identified in the milk of mothers receiving these drugs. The effects, if any, on the breast fed child have not been determined. If feasible, the use of O.C.s should be deferred until the infant has been weaned. **GENERAL WARNINGS:** 1. A careful medical and family history should be taken prior to the initiation of O.C.s. The pretreatment and periodic physical examinations should include special reference to blood pressure, breasts, abdomen and pelvic organs, including Papanicolaou smear and relevant laboratory tests. As a general rule, O.C.s should not be prescribed for longer than 1 year without another physical examination being performed. 2. Under the influence of estrogen-progestogen preparations, preexisting uterine leiomyomata may increase in size. 3. Patients with a history of psychic depression should be carefully observed and the drug discontinued if depression recurs to a serious degree. Patients becoming significantly depressed while taking O.C.s should stop the medication and use an alternate method of contraception in an attempt to determine whether the symptom is drug related. 4. O.C.s may cause some degree of fluid retention. They should be prescribed with caution, and only with careful monitoring, in patients with conditions which might be aggravated by fluid retention, such as convulsive disorders, migraine syndrome, asthma or cardiac, hepatic or renal insufficiency. 5. Patients with a past history of jaundice during pregnancy have an increased risk of recurrence of jaundice while receiving O.C. therapy. If jaundice develops in any patient receiving such drugs, the medication should be discontinued. 6. Steroid hormones may be poorly metabolized in patients with impaired liver function and should be administered with caution in such patients. 7. O.C. users may have disturbances in normal tryptophan metabolism which may result in a relative pyridoxine deficiency. The clinical significance of this is unknown. 8. Serum folate levels may be depressed by O.C. therapy. Since the pregnant woman is predisposed to the development of folate deficiency and the incidence of folate deficiency increases with increasing gestation, it is possible that if a woman becomes pregnant shortly after stopping O.C.s, she may have a greater chance of developing folate deficiency and complications attributed to the deficiency. 9. The pathologist should be advised of O.C. therapy when relevant specimens are submitted. 10. Certain endocrine and liver function tests and blood components may be affected by estrogen-containing O.C.s. For example: a. Increased sulfolipid retention. b. Increased prothrombin and factors VII, VIII, IX, and X; decreased antithrombin III; increased norepinephrine-induced platelet aggregability. c. Increased thyroid binding globulin (TBG) leading to increased circulating total thyroid hormone, as measured by protein-bound iodine (PBI), T4 by column, or T4 by radioimmunoassay. Free T3 resin uptake is decreased, reflecting the elevated TBG. Free T4 concentration is unaltered. d. Decreased pregnandiol excretion. e. Reduced response to metyrapone test. **DRUG INTERACTIONS:** O.C.s may be rendered less effective by virtue of drug interaction with rifampin, isoniazid, ampicillin, tetracycline, neomycin, penicillin V, chloramphenicol, sulfonamides, nitrofurantoin, barbiturates, phenytoin, primidone, analgesics, tranquilizers, and antimigraine preparations. O.C.s may alter the effectiveness of other types of drugs, such as oral anticoagulants, anticonvulsants, tricyclic antidepressants, antihypertensive agents (e.g., guanethidine), vitamins and hypoglycemic agents. **CARCINOGENESIS:** See Warnings, **PREGNANCY** category A. See Contraindications and Warnings, **NURSING MOTHERS** See Warnings, **Adverse Reactions** An increased risk of the following serious adverse reactions has been associated with the use of O.C.s (see Warnings): thrombophlebitis, thrombosis, pulmonary embolism, coronary thrombosis, cerebral thrombosis, mesenteric thrombosis, liver tumors, cerebral hemorrhage, hypertension, gall bladder disease, congenital anomalies, neuro-ocular lesions, e.g., retinal thrombosis and optic neuritis. The following adverse reactions have been reported in patients receiving O.C.s and are believed to be drug related: bleeding irregularities (breakthrough bleeding, spotting, missed menses) during treatment; amenorrhea after treatment; abnormal uterine bleeding patterns (nausea, vomiting, bloating, abdominal cramps); dysmenorrhea, infertility after discontinuance of treatment; edema, chloasma or melasma which may persist after drug is discontinued; breast changes (tenderness, enlargement, and secretion); intolerance to contact lenses; change in corneal curvature (steepening) change in weight (increase or decrease); change in cervical erosion and cervical secretion; possible diminution in lactation when given immediately postpartum; cholestatic jaundice; migraine; increase in size of uterine leiomyomata; rash (allergic); mental depression; reduced tolerance to carbohydrates; vaginal candidiasis; prolactin-secreting pituitary tumors. The following adverse reactions have been reported in users of O.C.s and the association has been neither confirmed nor refuted: premenstrual-like syndrome, cataracts, changes in libido, chorea, changes in appetite, cystitis-like syndrome, headache, nervousness, dizziness, hirsutism, loss of hair, erythema multiforme, erythema nodosum, hemorrhagic eruption, vaginitis, porphyria, impaired renal function. **Information for the Patient (See Patient Package Insert)**



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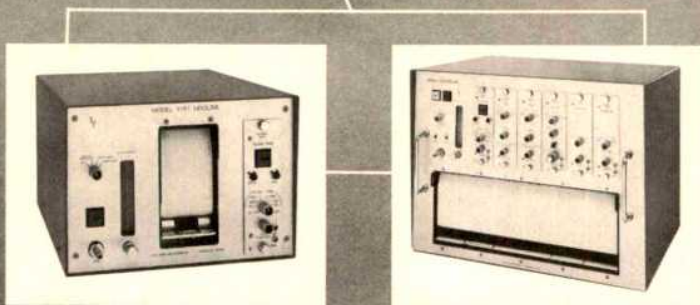
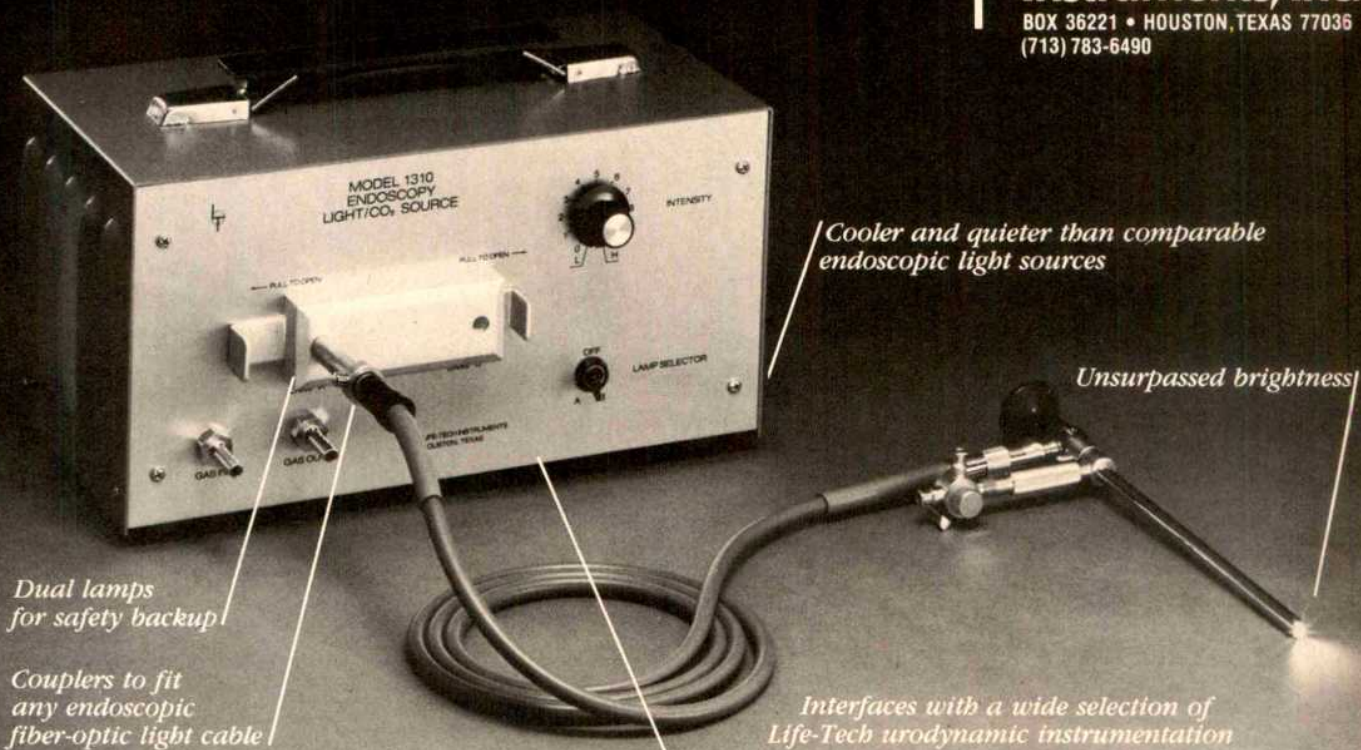
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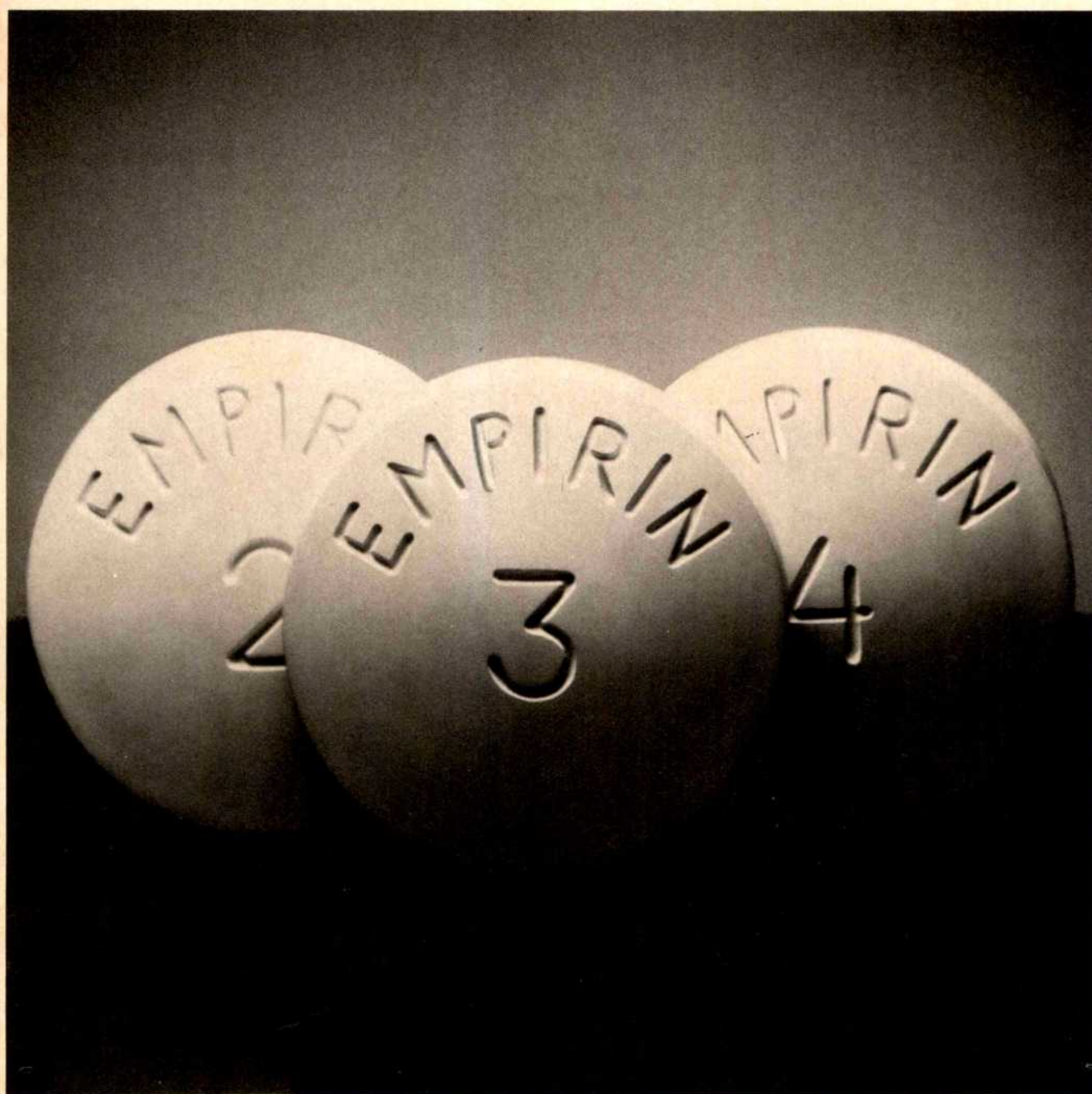


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## Contents

February 1

1981

## Transactions of the Thirty-sixth Annual Meeting of the Society of Obstetricians and Gynaecologists of Canada

- Significance of intrauterine adhesions detected hysteroscopically in eumenorrheic infertile women and role of antecedent curettage in their formation** 239

Patrick J. Taylor, M.B., M.R.C.O.G., F.R.C.S.(C.), David C. Cumming, M.B., M.R.C.O.G., F.R.C.S.(C.), and Peter J. Hill, M.B., M.R.C.O.G.  
Calgary, Alberta, Canada

- A protein-binding radioassay for 6 $\beta$ -hydroxycortisol: Detection in pregnancy urine and amniotic fluid** 243

S. J. Bowler-Wong, D. M. Hay, and F. L. Lorscheider  
Calgary, Alberta, Canada

(Contents continued on page 7)

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**Contraindications:** Douching is not recommended during pregnancy. VAGISEC Liquid Douche and VAGISEC PLUS Suppositories are spermicidal and should not be used on days when the patient is trying to conceive. **Precautions:** *Therapeutic Treatment:* Recurrence of vaginitis often indicates extravaginal foci or infection in cervical, vestibular and urethral glands, etc., or reinfection by the sexual partner. During treatment, patient should refrain from intercourse or the partner should wear a prophylactic. To prevent flare-ups after completion of therapy and for continued vaginal cleanliness, the patient is advised to continue with VAGISEC Liquid as a regular douche, but no more than twice weekly unless otherwise directed by physician. **Side Effects:** No significant side effects reported to date. Any minor irritation is alleviated by discontinuance of douche for a single day. **Administration and Dosage:** *Office Treatment (Adult):* For the treatment of vaginal trichomoniasis and mixed vaginal infections, best results are obtained by scrubbing the vagina with VAGISEC Liquid, diluted 1:100, by the physician in the office; 3 scrubs the first week, 2 the second. A suppository is inserted after each office scrub in the Liquid/Suppository regimen. Patient should be instructed to continue treatment at home. *Home Treatment:* Liquid/Suppository regimen: In all instances, the combination douche and suppositories are recommended for home use. The usual home treatment is VAGISEC Liquid Douche in the morning, followed by a VAGISEC PLUS Suppository inserted deep in the vagina and a second suppository inserted in like manner at bedtime, daily, for a period of 14 days. For those physicians who feel it important to treat the patient through the complete menstrual cycle, the Liquid/Suppository regimen may be continued throughout the month. It is advisable to continue treatment through two menstrual periods. Treatment should be omitted the night and morning preceding an office visit. Patients should be reexamined 3 days after home treatment has been discontinued. The full course of therapy must be completed to eliminate the infecting microorganisms from the vagina. In chronic or stubborn cases, therapy may be repeated with no interval between courses. During treatment, patient should refrain from intercourse, or the partner should wear a prophylactic. No patient should be considered cured until vaginal smears or cultures, taken at monthly intervals for 3 months following treatment, show absence of trichomonads and a return to the normal vaginal flora. **How Supplied:** VAGISEC Liquid—4 oz. plastic bottle. VAGISEC PLUS Suppositories—box of 28 3-gram suppositories, foil-wrapped, with inserter. Federal law prohibits dispensing VAGISEC PLUS suppositories without prescription. More detailed professional information available on request.

\*SEM photographs from S.T.E.M. Laboratories, Inc., Kansas City, Missouri 64127

1. Davis, C. H.: JAMA 157:126, 1955. 2. Davis, C. H. and Grand, C. G.: Am. J. Obst. & Gynec.: 68:559, 1954. 3. Abruzzi, W. A.: West. Med. 3:44, 1962.

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SEM photo\* shows trichomonad before treatment with VAGISEC Liquid Douche (orig. mag. 2KX)



15 seconds after treatment, photo shows cell wall destruction and loss of flagella (orig. mag. 5KX)

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|                                                                                                                                                                                             |     |
|---------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|-----|
| <b>6<math>\beta</math>-Hydroxycortisol levels in maternal urine of pregnancies complicated by prematurity</b>                                                                               | 250 |
| D. C. Cumming, E. J. Love, and F. L. Lorscheider<br><i>Calgary, Alberta, Canada</i>                                                                                                         |     |
| <b>Qualitative amniotic fluid volume determination by ultrasound: Antepartum detection of intrauterine growth retardation</b>                                                               | 254 |
| Frank A. Manning, M.D., Lyndon M. Hill, M.D., and Lawrence D. Platt, M.D.<br><i>Winnipeg, Manitoba, Canada, Rochester, Minnesota, and Los Angeles, California</i>                           |     |
| <b>Phosphatidylglycerol determination on amniotic fluid 10,000 <math>\times</math> g pellet in the prediction of fetal lung maturity</b>                                                    | 259 |
| A. E. Bent, M.D., J. H. Gray, R.N., E. R. Luther, F.R.C.S.(C.), M. Oulton, Ph.D., and L. J. Peddle, F.R.C.S.(C.)<br><i>Halifax, Nova Scotia, Canada</i>                                     |     |
| <b>Hyperprolactinemia—A significant factor in female infertility</b>                                                                                                                        | 264 |
| Jeremy V. Kredentser, B.A., M.D., Christopher F. Hoskins, M.B., Ch.B., F.R.C.S.(C.), and Josef Z. Scott, M.B., Ch.B., F.A.C.O.G., F.R.C.S.(C.)<br><i>Edmonton, Alberta, Canada</i>          |     |
| <b>Transvaginal retropubic urethropexy</b>                                                                                                                                                  | 268 |
| G. J. Quigley, M.D., F.R.C.S.(C.), F.R.C.O.G., and S. K. King, M.D.<br><i>Hamilton, Ontario, Canada</i>                                                                                     |     |
| <b>Urodynamic studies in female patients with multiple sclerosis</b>                                                                                                                        | 273 |
| R. Peter Beck, M.D., C.M., F.A.C.O.G., F.R.C.O.G., F.R.C.S.(C.), K. G. Warren, B.A., M.D., F.R.C.P.(C.), and P. Whitman, R.N.<br><i>Edmonton, Alberta, Canada</i>                           |     |
| <b>Acute adolescent menorrhagia</b>                                                                                                                                                         | 277 |
| E. Anne Claessens, M.B., B.S., F.R.C.S.(C.), and Carol A. Cowell, M.D., F.R.C.S.(C.)<br><i>Toronto, Ontario, Canada</i>                                                                     |     |
| <b>Management of congenital absence of the vagina</b>                                                                                                                                       | 281 |
| F. E. Bryans, M.D., F.R.C.S.(C.)<br><i>Vancouver, British Columbia, Canada</i>                                                                                                              |     |
| <b>Endometrial neoplasia—Screening the high-risk patient</b>                                                                                                                                | 285 |
| Joseph H. Cramer, M.D.C.M., F.R.C.S.(C.), and Raymond J. Osborne, M.D., F.R.C.S.(C.)<br><i>Toronto, Ontario, Canada</i>                                                                     |     |
| <b>Postradiation ureteral obstruction: A reappraisal</b>                                                                                                                                    | 289 |
| David Muram, M.D., Harry Oxorn, M.D., R. H. Curry, M.D., F.R.C.P.(C.), P. Drouin, M.D., F.A.C.O.G., F.R.C.S.(C.), and Jack H. Walters, M.D., F.R.C.S.(C.)<br><i>Ottawa, Ontario, Canada</i> |     |

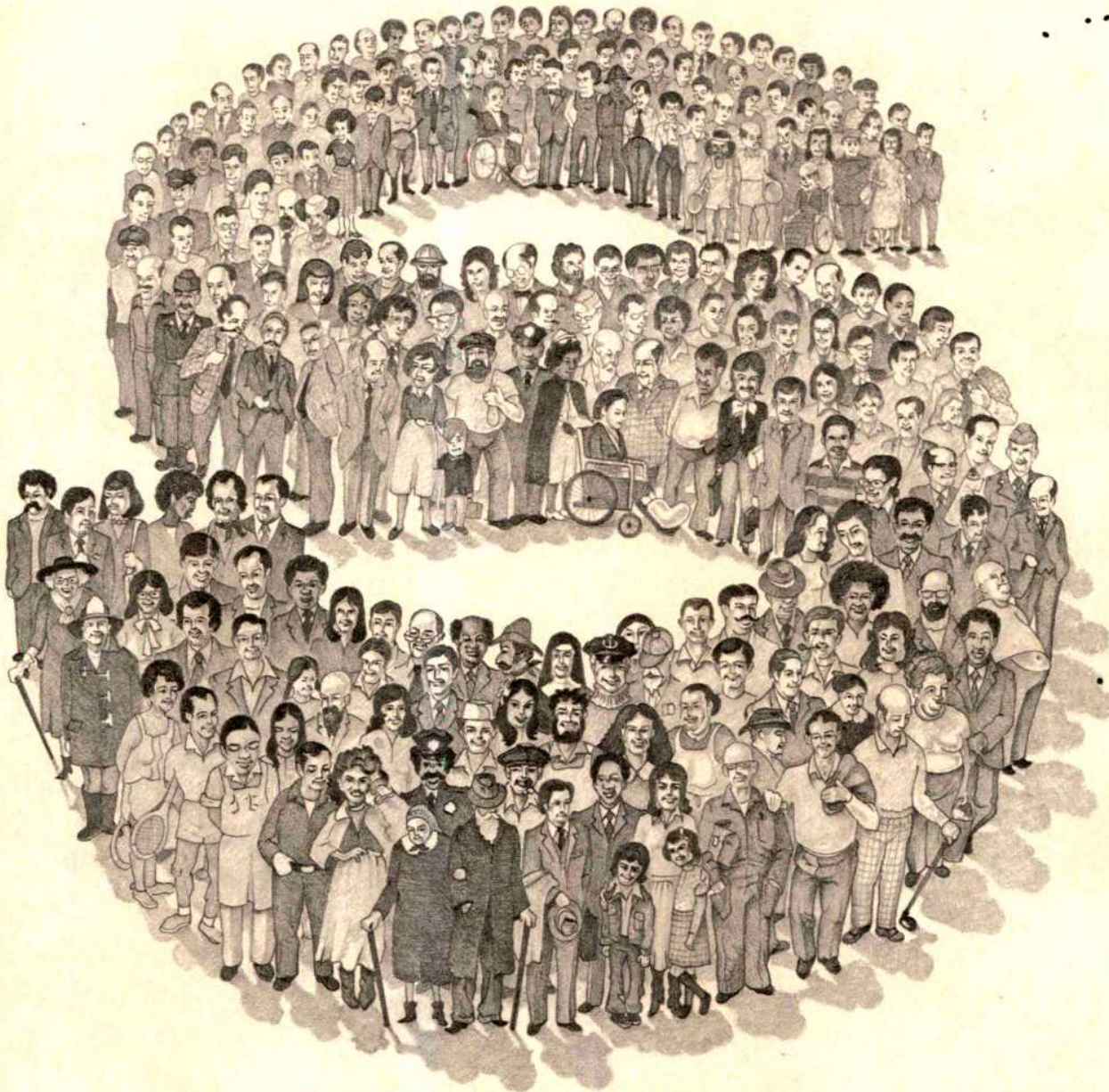
(Contents continued on page 9)



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# Contents continued from page 7

|                                                                                                                                                                                      |     |
|--------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|-----|
| <b>Risk factors associated with infection following cesarean section</b>                                                                                                             | 294 |
| P. A. Hawrylyshyn, M.D., M.Sc., P. Bernstein, M.D., F.R.C.S.(C.), and<br>F. R. Papsin, M.D., F.R.C.S.(C.)<br><i>Toronto, Ontario, Canada</i>                                         |     |
| <b>The prediction of intrapartum fetal metabolic acidosis by fetal heart rate monitoring</b>                                                                                         | 299 |
| J. A. Low, M. J. Cox, E. J. Karchmar, M. J. McGrath, S. R. Pancham, and W. N. Piercy<br><i>Kingston, Ontario, Canada</i>                                                             |     |
| <b>The effect of maternal, labor, and fetal factors upon fetal heart rate during the intrapartum period</b>                                                                          | 306 |
| J. A. Low, M. J. Cox, E. J. Karchmar, M. J. McGrath, S. R. Pancham, and W. N. Piercy<br><i>Kingston, Ontario, Canada</i>                                                             |     |
| <b>Squamous cell carcinoma of the vagina following hysterectomy</b>                                                                                                                  | 311 |
| G. C. E. Stuart, M.D., H. H. Allen, M.D., and R. J. Anderson, M.D.<br><i>London, Ontario, Canada</i>                                                                                 |     |
| <b>Molar pregnancy in British Columbia: Estimated incidence and postevacuation regression patterns of the beta subunit of human chorionic gonadotropin</b>                           | 316 |
| Basil Ho Yuen, M.B., F.R.C.S.(C.), and Wendy Cannon, B.Sc.<br><i>Vancouver, British Columbia, Canada</i>                                                                             |     |
| <b>Puerperal febrile complications and cervical flora following elective manual exploration of the uterus</b>                                                                        | 320 |
| Eduardo Berger, M.D., Martin S. Gillieson, M.B., F.R.C.S.(C.), and<br>Jack H. Walters, M.D., F.R.C.S.(C.)<br><i>Ottawa, Ontario, Canada</i>                                          |     |
| <b>Effects of synthetic glucocorticoid administration on human fetal breathing movements at 34 to 35 weeks' gestational age</b>                                                      | 324 |
| John Patrick, M.D., John Challis, Ph.D., Karen Campbell, M.E.Sc., Lesley Carmichael, B.Sc.,<br>Bryan Richardson, M.D., and Gerald Tevaarwerk, M.D.<br><i>London, Ontario, Canada</i> |     |
| <b>Dilation and evacuation: A preferred method of midtrimester abortion</b>                                                                                                          | 329 |
| K. I. Cadesky, M.D., E. Ravinsky, M.D., and E. R. Lyons, M.D.<br><i>Toronto, Ontario, Canada</i>                                                                                     |     |
| <b>Factors influencing morbidity in termination of pregnancy</b>                                                                                                                     | 333 |
| Christopher R. Harman, M.D., David G. Fish, Ph.D., and John E. Tyson, M.D.<br><i>Winnipeg, Manitoba, Canada</i>                                                                      |     |
| <b>Loss of diurnal rhythm in plasma estrone, estradiol, and estriol in women treated with synthetic glucocorticoids at 34 to 35 weeks' gestation</b>                                 | 338 |
| John Challis, Ph.D., John Patrick, M.D., Bryan Richardson, M.D., and Gerald Tevaarwerk, M.D.<br><i>London, Ontario, Canada</i>                                                       |     |

(Contents continued on page 11)



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## Contents continued from page 9

|                                                                                                                                        |     |
|----------------------------------------------------------------------------------------------------------------------------------------|-----|
| <b>Effects of external physical stimulation on fetuses near term</b>                                                                   | 344 |
| Bryan Richardson, M.D., Karen Campbell, M.E.Sc., Lesley Carmichael, B.Sc., and<br>John Patrick, M.D.<br><i>London, Ontario, Canada</i> |     |
| <b>Demonstration of novel compounds in human fetal tissues and a consideration of<br/>their possible role in parturition</b>           | 353 |
| Beverley E. Pearson Murphy<br><i>Montreal, Quebec, Canada</i>                                                                          |     |
| <b>Adrenal responses to prostaglandin E<sub>2</sub> in newborn lambs</b>                                                               | 359 |
| George D. Carson and John R. G. Challis<br><i>Calgary, Alberta, and London, Ontario, Canada</i>                                        |     |

## Items

|              |     |
|--------------|-----|
| <b>Items</b> | 364 |
|--------------|-----|

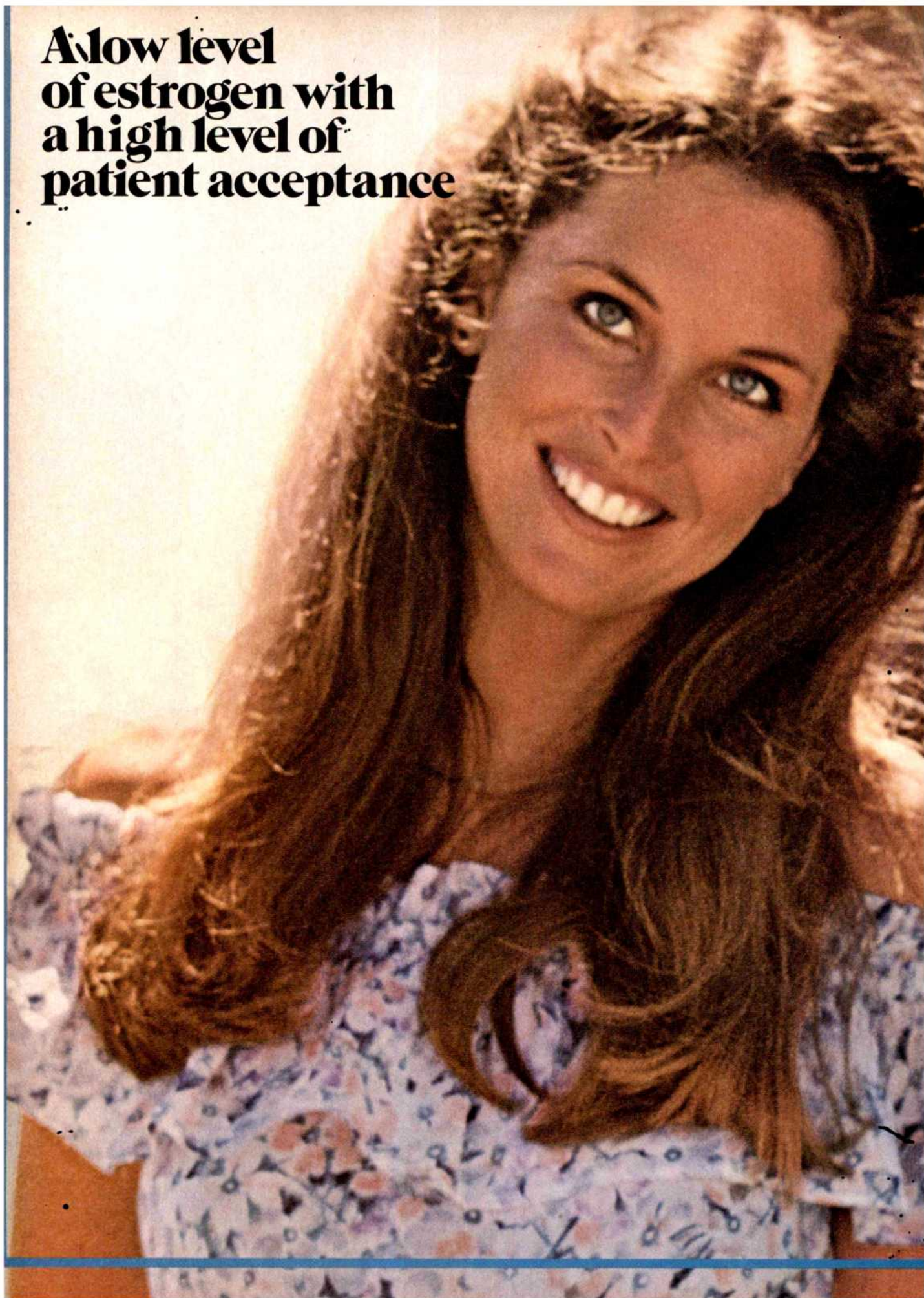
*Information for authors on page 21*

*Index to advertisers on page 37*

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**Contraindications**—Thrombophlebitis, thromboembolic disorders, cerebrovascular disease, myocardial infarction or coronary artery disease, or past history of these, known or suspected breast carcinoma, estrogen-dependent neoplasia, or pregnancy, undiagnosed abnormal vaginal bleeding, markedly impaired liver function, past or present, benign or malignant liver tumors.

## Warnings

**Cigarette-smoking increases risk of serious cardiovascular side effects from OC use. Risk increases with age and heavy smoking (15 or more cigarettes/day) and is quite marked in women over 35. OC users should be strongly advised not to smoke.**

OCs are associated with increased risk of several serious conditions including venous and arterial thromboembolism, thrombotic and hemorrhagic stroke, myocardial infarction, visual disorders, hepatic tumors, gallbladder disease, hypertension, fetal abnormalities. Practitioners should be familiar with the available information relating to these and other risks.

**1. Thromboembolic disorders and other vascular problems**—Increased risk of thromboembolic and thrombotic disease associated with OCs is well established. A British study showed increased relative risk for fatal venous thromboembolism; 2 British and 3 U.S. studies showed increased relative risk for nonfatal venous thromboembolism. Two U.S. studies showed increased relative risk for stroke, not shown in prior British studies. It was estimated OC users were 2–11 times more likely than nonusers to manifest these diseases without evident cause. In a British study of idiopathic deep vein thrombosis and pulmonary embolism, projected hospitalization rates for ages 15–40 were 47/year/100,000 users and 5 for nonusers. In a British mortality study, overall excess mortality from pulmonary embolism or stroke was 1.3–3.4 deaths/year/100,000 users and increased with age. **Cerebrovascular Disorders**—In a U.S. study of stroke with and without predisposing causes, risk of hemorrhagic stroke was estimated as 2 times greater and thrombotic stroke 4.0–9.5 times greater in users. Mortality trends in 21 countries indicate that, since OC introduction, changes in mortality from nonhemorrhagic cardiovascular disease were associated with changes in the prevalence of OC use. The Royal College of GPs reported on its prospective study comparing users and nonusers: "A statistically significant higher rate of reporting of cerebrovascular accidents in Takers is evident, but the numbers are too small to justify an estimation of the degree of risk." They reported more superficial and deep vein thrombosis in users. A 1977 analysis showed a 5-fold increased mortality in users from circulatory diseases, mainly myocardial infarction (MI) and hemorrhagic stroke. Excess mortality was associated with age, smoking and duration of use. In 1976 a British Family Planning Association study found a highly significant association between OC use and stroke though total numbers were small; the increased risk for venous thrombosis and pulmonary embolism was about 4-fold and statistically highly significant. Circulatory disease mortality was significantly greater in users. **Myocardial Infarction**—An increased risk of MI associated with OC use was reported in Britain, as previously suspected. The morbidity study found the greater the number of risk factors for coronary artery disease (smoking, hypertension, hypercholesterolemia, obesity, diabetes, history of pre-eclampsia/toxemia) the higher the MI risk, regardless of OC use. OCs were considered an additional risk factor. The excess rate of fatal MI in Britain was about 3.5/year/100,000 users aged 30–39 and 20 for ages 40–44. (These are based on British vital statistics with acute MI death rates 2–3 times less than the U.S. Similar estimates for U.S. are 25.7 for users aged 30–39 with predispositions vs. 15 without; for ages 40–44, 86.2 vs. 5.1.) Excess hospitalization rate for nonfatal MI in Britain was about 3.5/year/100,000 users aged 30–39 and 47 for ages 40–44. Smoking is a major predisposing condition to MI. In terms of relative risk, nonsmoking OC users are twice as likely to have fatal MI as nonsmoking nonusers. Smoking users have a 5-fold greater risk of fatal MI as nonsmoking users, but a 10–12-fold greater risk than nonsmoking nonusers. Amount of smoking is an important factor in determining importance of these risks, give serious consideration to rates in table below. Importance of other predispositions cited above in determining relative and absolute risks has not been quantified; it is likely the same synergism exists, but perhaps to a lesser extent.

| Estimated Mortality Rate from Myocardial Infarction per 100,000 Women/Year |            |          |            |          |
|----------------------------------------------------------------------------|------------|----------|------------|----------|
|                                                                            | Aged 30–39 |          | Aged 40–44 |          |
|                                                                            | Users      | Nonusers | Users      | Nonusers |
| All smokers                                                                | 10.2       | 2.6      | 62.0       | 15.9     |
| ≥15 cigarettes daily                                                       | 13.0       | 5.1      | 78.7       | 31.3     |
| <15 cigarettes daily                                                       | 4.7        | 0.9      | 28.6       | 5.7      |
| Nonsmokers                                                                 | 1.8        | 1.2      | 10.7       | 7.4      |
| Smokers and nonsmokers                                                     | 5.4        | 1.9      | 32.8       | 11.7     |

**Risk of Dose**—Reports of thromboembolism after use of OCs with  $\geq 50$  mcg. estrogen received by drug safety committees in Britain, Sweden and Denmark were compared with the distribution expected from market research sales estimates. A positive correlation was found between estrogen dose and reporting of thromboembolism, including coronary thrombosis, in excess of that predicted by sales. OCs with  $\geq 100$  mcg. estrogen were associated with higher thromboembolic risk than those with 50–80 mcg. The authors suggested that estrogen quantity may not be the sole factor. Any influence of progestogens was not considered, which may be responsible for certain discrepancies in the data. No significant differences were noted between OCs containing the same estrogen dose nor between ethinyl estradiol and mestranol. Studies on thromboembolic risk with progestogen-only OCs have not been done. Cases have been reported for these OCs and they are not presumed free of excess risk. In a U.S. case-controlled study, relative risk of OC use 1 month before hospitalization for various types of thromboembolism was calculated. If relative estrogenic potency and possible influence of different progestogens are ignored, OCs may be divided into those with  $<100$  mcg. and those with  $\geq 100$  mcg. estrogen. For all cases combined, larger doses were associated with only slightly higher relative risk for idiopathic cases relative risk was doubled with larger doses, but confidence limits overlapped and differences were not significant. Apparently there was no increased risk for cases with predispositions. Thromboembolic risk in users and nonusers increases with age. OCs have been considered an independent risk factor. **Excess Mortality from Circulatory Diseases**—A U.K. prospective study estimated annual mortality from circulatory diseases for OC users and nonusers according to age, smoking habits and duration of use. Excess mortality was 20/100,000 users (5 for ages 15–34, 33 for ages 35–44, 140 for ages 45–49), the risk being concentrated in older women, long-term users, and smokers. Interrelationships of age, smoking and duration of use could not be examined, nor could effects of continuous vs. intermittent use. Though circulatory mortality was increased 10-fold in users of  $>5$  years, all deaths were in women over age 34. Until more under-35 users of 5+ continuous years are available, size of relative risk for this age group cannot be given. Risk of circulatory disease may have persisted after discontinuation of OCs. One study estimated risk of death with various contraceptive methods, each estimate including risk of method (e.g., thromboembolism for OCs) plus risk due to pregnancy or abortion in cases of method failure. This latter risk varies with effectiveness. It concluded mortality with all methods is low and below that of childbirth, except for OC users over 40 who smoke. Heavy smokers have double the risk of smokers as a class, light smokers about half. Mortality associated with OC use in nonsmokers over 40 is higher than with any other method in that age group. Lowest mortality is with the condom or diaphragm backed up by early legal abortion. Risk of thromboembolic and thrombotic disease associated with OCs increases with age after 30 and, for MI, is further increased by hypertension, hypercholesterolemia, obesity, diabetes, history of pre-eclampsia/toxemia, and especially cigarette smoking. Based on current data, the chart gives gross estimates of risk of death from circulatory disorders associated with OCs:

| Risk is: A—very high, B—high, C—moderate, D—low. | Age: | <30 | 30–39 | ≥40 |
|--------------------------------------------------|------|-----|-------|-----|
| Heavy smokers (≥15/day)                          |      | C   | B     | A   |
| Light smokers (<15/day)                          |      | D   | C     | B   |
| Nonsmokers, no predispositions                   |      | D   | C     | B   |
| Nonsmokers, with predispositions                 |      | D   | C     | B   |

Physician and patient should be alert to earliest manifestations of thromboembolic and thrombotic disorders (e.g., thrombophlebitis, pulmonary embolism, cerebrovascular insufficiency, coronary artery disease, myocardial infarction, retinal thrombosis, mesenteric thrombosis). Should any of these occur or be suspected, discontinue OCs immediately. A 4–7-fold increased risk of postoperative thromboembolism was reported in users; if feasible discontinue OCs at least 4 weeks before elective surgery or during prolonged immobilization and do not resume until at least 4 weeks postoperative. Thromboembolic risk may be increased for users with blood types A, B or AB. **2. Ocular lesions**—Neuro-ocular lesions such as optic neuritis or retinal thrombosis have been associated with OCs. Discontinue if there is unexplained, gradual or sudden, partial or complete loss of vision; proptosis or diplopia; papilledema; or evidence of retinal vascular lesions. Institute diagnostic and therapeutic measures. **3. Carcinoma**—Long-term estrogen in certain animals increases carcinomas and/or nonmalignant neoplasms, such as those of breast, uterus, cervix, vagina, ovary, liver, pituitary. Some

synthetic progestogens, none currently in OCs, increase incidence of benign and malignant mammary nodules in dogs. There is now evidence that estrogens increase risk of human endometrial carcinoma. In several case-controlled studies increased relative risk (2.2–13.9 times) associated endometrial carcinoma with prolonged estrogen for menopausal symptoms. Risk was independent of other known risk factors and depended on duration of use and dose. This is supported by increased endometrial cancer incidence since 1969 in 8 U.S. areas with cancer-reporting systems, which may be related to rapidly expanding estrogen use. There is no evidence that natural estrogens are more or less hazardous than synthetics at equi-estrogenic doses. Of the first 30 cases of endometrial carcinoma in OC users under 40, those without predispositions (e.g., irregular bleeding before OCs, polycystic ovaries) occurred nearly always with no longer-marketed sequential OCs. No statistical association is reported suggesting increased endometrial cancer with combination or progestogen-only OCs, though individual cases have been reported. Studies show no increased breast cancer with OCs or estrogens. However, greater risk was noted in OC users with benign breast disease or long-term (2–4 years) use. Risk of breast cancer with menopausal estrogens rose and further increased with duration of follow-up. Decreased benign breast tumors in OC users is well known. Some studies suggested increased risk of cervical dysplasia, erosion and carcinoma in long-term users; cause and effect is not established. Cervical microglandular hyperplasia has been reported. In summary, there is no confirmed evidence from human studies of increased cancer risk with OCs. Close surveillance is, however, essential. In undiagnosed persistent or recurrent abnormal vaginal bleeding, consider nonfunctional causes and take appropriate diagnostic measures to rule out malignancy. Carefully monitor users with a strong family history of breast cancer or with breast nodules, fibrocystic disease, recurrent cystic mastitis, abnormal mammograms, cervical dysplasia. **4. Hepatic adenomas, hepatomas, hamartomas, regenerating nodules, focal nodular hyperplasia, hemangiomas, hepatocellular carcinoma, etc.** have been associated with OCs. In 1 study OC formulations with high "hormonal potency" were associated with higher risk. Though benign these hepatic lesions may rupture and cause fatal hemorrhage in short- or long-term users. Two studies related risk to duration of use, being much greater after  $\geq 4$  years. Consider such lesions in users with abdominal pain, tenderness, mass or shock. About 25% present with abdominal masses; up to half with acute hemoperitoneum. Routine radiologic and lab studies may not be helpful; liver scans may show a focal defect. Hepatic arteriography or CAT may be useful in diagnosis. Hepatocellular carcinoma was noted in a few users. OCs are contraindicated in case of past or present, benign or malignant liver tumors. **5. Use in or immediately preceding pregnancy, birth defects, malignancy in offspring**—Estrogens or progestogens during early pregnancy may seriously damage offspring. Females exposed in utero to DES and other nonsteroidal estrogens may have increased risk of vaginal or cervical cancer. Though OCs may not enhance this risk, monitor such patients carefully. From 30–90% of DES-exposed women develop benign vaginal or cervical adenosis, which could be premalignant. Males so exposed may develop urogenital defects. Other estrogens may mimic DES. Reports relate fetal exposure to female sex hormones and congenital anomalies, e.g., multiple anomalies described by VACTERL for vertebral, anal, cardiac, tracheoesophageal, renal and limb defects. There appears preferential expression by males. There was a 4.7-fold increased risk of limb-reduction defects in infants exposed in utero to sex hormones (OCs, hormonal withdrawal pregnancy tests or treatment for threatened abortion). Some exposures were very short, being only a few days. Cardiovascular defects in children exposed to female hormones, including OCs, during early pregnancy occurred at 18.2/1000 births, compared to 7.8 for those unexposed ( $P<0.05$ ). Female sex hormones were used for threatened or habitual abortion, but there is considerable evidence estrogens are ineffective and no evidence progestogens are effective. Triply and possibly other polyploidy may be increased in abortions from women becoming pregnant soon post-OC. These almost always abort spontaneously. It is unknown if spontaneous abortion is increased soon after stopping OCs. Safety of OCs in pregnancy has not been demonstrated. Rule out pregnancy in using OCs and always consider if withdrawal bleeding does not occur. If 2 consecutive periods are missed rule out pregnancy before continuing. If user deviates from regimen, consider pregnancy at first missed period and withhold OCs. If pregnancy is confirmed, apprise patient of potential fetal risks and discuss continuation of pregnancy. Users discontinuing for pregnancy should use alternative contraception for 3 months before attempting to conceive, though this is not based on precise information. Do not use progestogen-only or progestogen-estrogen drugs as pregnancy tests. **6. Gallbladder disease risk**, surgically confirmed, appeared after 2 years' use and doubled after 4–5 years in 1 study; it appeared in 6–12 months' use in another. **7. Carbohydrate & lipid metabolism**—Decreased glucose tolerance occurs in a significant number of users; carefully observe diabetic and prediabetic patients. Triglycerides and total phospholipids may increase. **8. Elevated blood pressure (BP)** occurs in some users in a few months, and degree may relate to progestogen dose. Hypertension prevalence in users is no higher than in nonusers in the first year, but increases with longer exposure; in the 5th year it is 2½–3 times the first year. Age is strongly correlated with hypertension in users. Those with history of elevated BP (hypertension), pre-existing renal disease, history of toxemia or elevated BP in pregnancy, familial tendency to hypertension or its consequences, or history of excessive weight gain or fluid retention during menstrual cycles may be more likely to develop elevated BP on OCs and thus should be monitored closely. Even though elevated BP may be in the "normal" range, do not ignore clinical implications. Follow closely, particularly those with the risk factors for cardiovascular disease or stroke. High BP may or may not persist after stopping OCs. **9. Headache**—Onset or exacerbation of migraine or development of headache of a new pattern that is recurrent, persistent or severe requires stopping OCs and evaluating cause. **10. Bleeding irregularities**—Breakthrough bleeding (BTB), spotting and amenorrhea are frequent reasons for discontinuing. In BTB, as in all cases of irregular bleeding, consider nonfunctional causes. In undiagnosed persistent or recurrent bleeding, rule out pregnancy or malignancy. If pathology is excluded, time or change to another product may suffice. Change to a higher estrogen OC only when necessary as this may increase thromboembolic risk. Those with a history of oligomenorrhea or secondary amenorrhea or without established regular cycles may be anovulatory or amenorrheic after discontinuing. Those with such problems should be so informed and other methods encouraged. Post-use anovulation, possibly prolonged, may occur in women without previous irregularities. Higher incidence of galactorrhea and of pituitary tumors (e.g., adenomas) has been associated with postpill amenorrhea. One study reported 16-fold increased prevalence of pituitary prolactin-secreting tumors in patients with postpill amenorrhea when galactorrhea was present. **11. Ectopics**—In failures with combination OCs, the ratio of ectopic to intrauterine pregnancies is no higher than in nonusers. There may be an increased risk of ectopics after discontinuing. **12. Breast feeding**—OCs may decrease quantity and quality of milk. A small fraction of OC steroids appears in milk. Effects if any on the child are unknown. If feasible defer OCs until after weaning. **13. Infertility** may occur in women discontinuing OCs, which appears independent of duration of use. While impairment diminishes with time, there is still an appreciable difference in nulliparous but not parous women after 30 months; difference is negligible after 42 months.

**Precautions**—Conduct complete history and physical prior to OC use and annually thereafter. Include breasts, abdomen and pelvis, with BP and lab tests. Pre-existing uterine leiomyomata may enlarge. Mental depression may occur, with positive history, observe carefully and discontinue if serious recurrence. Fluid retention may occur; prescribe cautiously and monitor carefully in convulsive disorders, migraine, asthma, or cardiac, hepatic or renal dysfunction. History of jaundice in pregnancy increases risk of recurrence on OCs; observe carefully and discontinue if jaundice occurs. Steroids may be poorly metabolized in liver dysfunction and should be given cautiously. Tryptophan metabolism may be disturbed and result in pyridoxine deficiency; megaloblastic anemia has been reported. Serum folate may be depressed and may be exacerbated in post-OC pregnancy. Advise pathologist of OC use when submitting specimens. Endocrine and liver function tests may be altered; repeat abnormal tests after stopping for 2 months. These have been observed: Increased BSP retention and other abnormalities in liver function tests; increased prothrombin and coagulation factors VII, VIII, IX, X, decreased antithrombin III, increased platelet aggregability; increased thyroid-binding globulin leading to increased circulating total thyroid hormone (PBI, T<sub>4</sub>); decreased T<sub>3</sub> uptake, unaltered free T<sub>4</sub>; decreased pregnanediol excretion; reduced methyrapone response; increased blood transferrin, corticosteroid, triglyceride, phospholipid, reduced serum folate; impaired glucose tolerance; altered plasma trace minerals; influence of prolonged OC use on pituitary, ovarian, adrenal, hepatic, or uterine function, or on immune response is not established. OCs may mask onset of climacteric. Information for patients—see patient labeling. **Drug interactions**—OC effectiveness may be decreased and more BTB occur with rifampicin, isoniazid, ampicillin, neomycin, penicillin V, chloramphenicol, sulfonamides, nitrofurantoin, barbiturates, phenytoin, primidone, phenylbutazone, analgesics, tranquilizers, anticholinergics, OCs may alter effectiveness of drugs such as oral anticoagulants, anticonvulsants, tricyclic antidepressants, antihypertensives, vitamins, hypoglycemics.

**Adverse reactions**—Increased risk of the following serious adverse reactions has been associated with OCs: thrombophlebitis and thrombosis; pulmonary embolism; myocardial infarction and coronary thrombosis; cerebral thrombosis; cerebral hemorrhage; hypertension; gallbladder disease; benign adenomas and other hepatic lesions; with or without intra-abdominal bleeding; congenital abnormalities. There is evidence of association between the following conditions and OCs, though confirmatory studies have not been done: mesenteric thrombosis; Budd-Chiari syndrome, neuro-ocular lesions, e.g., retinal thrombosis and optic neuritis. The following have been reported with OCs and are believed drug related: nausea, vomiting, gastrointestinal symptoms, breakthrough bleeding, spotting, change in menstrual flow, dysmenorrhea, amenorrhea during and after use, infertility post-OC, edema, chloasma or melasma, which may persist post-OC, breast changes (tenderness, enlargement, secretion), weight changes, change in cervical erosion and secretion, endocervical hyperplasia, lactation decrease, cholestatic jaundice, migraine, enlarged uterine leiomyomata, allergic rash, mental depression, reduced carbohydrate tolerance, vaginal candidiasis, change in corneal curvature (steepening), intolerance to contact lenses. The following have been reported and association has not been confirmed or refuted: premenstrual-like syndrome, cataracts, libido changes, chorea, appetite changes, cystitis-like syndrome, headache, paresthesia, nervousness, dizziness, auditory disturbances, rhinitis, fatigue, backache, hirsutism, scalp hair loss, erythema multiforme or nodosum, hemorrhagic eruption, itching, vaginitis, porphyria, impaired renal function, anemia, pancreatitis, hepatitis, colitis, gingivitis, lupus erythematosus, rheumatoid arthritis, pituitary tumors (e.g., adenoma) with amenorrhea and/or galactorrhea after use, endometrial, cervical and breast carcinoma.

**Acute overdosage**—Serious ill effects have not been reported after large acute doses of OCs in young children, but nausea and withdrawal bleeding might occur.

**Important note**—Complete prescribing information should be consulted prior to use and is available on request.

**SEARLE**

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Please see following page for brief summary of prescribing information.



## PREMARIN® Brand of Conjugated Estrogens Tablets, U.S.P.

### 1. ESTROGENS HAVE BEEN REPORTED TO INCREASE THE RISK OF ENDOMETRIAL CARCINOMA.

Three independent case control studies have reported an increased risk of endometrial cancer in postmenopausal women exposed to exogenous estrogens for more than one year.<sup>1-3</sup> This risk was independent of the other known risk factors for endometrial cancer. These studies are further supported by the finding that incidence rates of endometrial cancer have increased sharply since 1969 in eight different areas of the United States with population-based cancer reporting systems, an increase which may be related to the rapidly expanding use of estrogens during the last decade.<sup>4</sup> The three case control studies reported that the risk of endometrial cancer in estrogen users was about 4.5 to 13.9 times greater than in nonusers. The risk appears to depend on both duration of treatment<sup>1</sup> and on estrogen dose.<sup>2</sup> In view of these findings, when estrogens are used for the treatment of menopausal symptoms, the lowest dose that will control symptoms should be utilized and medication should be discontinued as soon as possible. When prolonged treatment is medically indicated, the patient should be reassessed on at least a semiannual basis to determine the need for continued therapy. Although the evidence must be considered preliminary, one study suggests that cyclic administration of low doses of estrogen may carry less risk than continuous administration.<sup>5</sup> It therefore appears prudent to utilize such a regimen. Close clinical surveillance of all women taking estrogens is important. In all cases of undiagnosed persistent or recurring abnormal vaginal bleeding, adequate diagnostic measures should be undertaken to rule out malignancy. There is no evidence at present that "natural" estrogens are more or less hazardous than "synthetic" estrogens at equiestrogenic doses.

### 2. ESTROGENS SHOULD NOT BE USED DURING PREGNANCY.

The use of female sex hormones, both estrogens and progestogens, during early pregnancy may seriously damage the offspring. It has been shown that females exposed in utero to diethylstilbestrol, a non-steroidal estrogen, have an increased risk of developing in later life a form of vaginal or cervical cancer that is ordinarily extremely rare.<sup>6,7</sup> This risk has been estimated as not greater than 4 per 1000 exposures.<sup>7</sup> Furthermore, a high percentage of such exposed women (from 30 to 90 percent) have been found to have vaginal adenosis.<sup>8-12</sup> Epithelial changes of the vagina and cervix. Although these changes are histologically benign, it is not known whether they are precursors of malignancy. Although similar data are not available with the use of other estrogens, it cannot be presumed they would not induce similar changes. Several reports suggest an association between intrauterine exposure to female sex hormones and congenital anomalies, including congenital heart defects and limb reduction defects.<sup>13-16</sup> One case control study<sup>16</sup> estimated a 4.7-fold increased risk of limb reduction defects in infants exposed in utero to sex hormones (oral contraceptives, hormone withdrawal tests for pregnancy, or attempted treatment for threatened abortion). Some of these exposures were very short and involved only a few days of treatment. The data suggest that the risk of limb reduction defects in exposed fetuses is somewhat less than 1 per 1000. In the past, female sex hormones have been used during pregnancy in an attempt to treat threatened or habitual abortion. There is considerable evidence that estrogens are ineffective for these indications, and there is no evidence from well controlled studies that progestogens are effective for these uses. If PREMARIN is used during pregnancy, or if the patient becomes pregnant while taking this drug, she should be apprised of the potential risks to the fetus, and the advisability of pregnancy continuation.

**DESCRIPTION:** PREMARIN (Conjugated Estrogens Tablets, U.S.P.) for oral administration contains a mixture of estrogens, obtained exclusively from natural sources, blended to represent the average composition of material derived from pregnant mares' urine. It contains estrone, equilin, and 17 $\alpha$ -dihydroequilin, together with smaller amounts of 17 $\alpha$ -estradiol, equilin, and 17 $\alpha$ -dihydroequilin as salts of their sulfate esters.

**INDICATIONS:** Based on a review of PREMARIN Tablets by the National Academy of Sciences—National Research Council and/or other information, FDA has classified the indications for use as follows:

#### Effective:

1. Moderate to severe vasomotor symptoms associated with the menopause. (There is no evidence that estrogens are effective for nervous symptoms or depression without associated vasomotor symptoms, and they should not be used to treat such conditions.)
2. Atrophic vaginitis.
3. Kraurosis vulvae.
4. Female hypogonadism.
5. Female castration.
6. Primary ovarian failure.
7. Breast cancer (for palliation only) in appropriately selected women and men with metastatic disease.
8. Prostatic carcinoma—palliative therapy of advanced disease.
9. Postpartum breast engorgement—Although estrogens have been widely used for the prevention of postpartum breast engorgement, controlled studies have demonstrated that the incidence of significant painful engorgement in patients not receiving such hormonal therapy is low and usually responsive to appropriate analgesic or other supportive therapy. Consequently, the benefit to be derived from estrogen therapy for this indication must be carefully weighed against the potential increased risk of puerperal thromboembolism associated with the use of large doses of estrogens.<sup>19</sup>

PREMARIN HAS NOT BEEN SHOWN TO BE EFFECTIVE FOR ANY PURPOSE DURING PREGNANCY AND ITS USE MAY CAUSE SEVERE HARM TO THE FETUS (SEE BOXED WARNING).

**"Probably" effective:** For estrogen deficiency-induced osteoporosis, and only when used in conjunction with other important therapeutic measures such as diet, calcium, physiotherapy, and good general health-promoting measures. Final classification of this indication requires further investigation.

**CONTRAINDICATIONS:** Estrogens should not be used in women (or men) with any of the following conditions: 1. Known or suspected cancer of the breast except in appropriately selected patients being treated for metastatic disease. 2. Known or suspected estrogen-dependent neoplasia. 3. Known or suspected pregnancy (See Boxed Warning). 4. Undiagnosed abnormal genital bleeding. 5. Active thrombophlebitis or thromboembolic disorders. 6. A past history of thrombophlebitis, thrombosis, or thromboembolic disorders associated with previous estrogen use (except when used in treatment of breast or prostatic malignancy).

**WARNINGS:** At the present time there is no satisfactory evidence that estrogens given to postmenopausal women increase the risk of cancer of the breast,<sup>17</sup> although a recent study has raised this possibility.<sup>18</sup> There is a need for caution in prescribing estrogens for women with a strong family history of breast cancer or who have breast nodules, fibrocystic disease, or abnormal mammograms. A recent study has reported a 2- to 3-fold increase in the risk of surgically confirmed gallbladder disease in women receiving postmenopausal estrogens.<sup>17</sup>

Adverse effects of oral contraceptives may be expected at the larger doses of estrogen used to treat prostatic or breast cancer or postpartum breast engorgement; it has been shown that there is an increased risk of thrombosis in men receiving estrogens for prostatic cancer and women for postpartum breast engorgement.<sup>19-22</sup> Users of oral contraceptives have an increased risk of diseases, such as thrombophlebitis, pulmonary embolism, stroke, and myocardial infarction.<sup>23-30</sup> Cases of retinal thrombosis, mesenteric thrombosis, and optic neuritis have been reported in oral contraceptive users.<sup>31,32</sup> An increased risk of postsurgery thromboembolic complications has also been reported in users of oral contraceptives.<sup>33,34</sup> If feasible, estrogen should be discontinued at least 4 weeks before surgery of the type associated with an increased risk of thromboembolism, or during periods of prolonged immobilization. Estrogens should not be used in persons with active thrombophlebitis, thromboembolic disorders, or in persons with a history of such disorders in association with estrogen use. They should be used with caution in patients with cerebral vascular or coronary

myocardial infarction, pulmonary embolism and thrombophlebitis, when doses of this size are used, any of the thromboembolic and thrombotic adverse effects should be considered a clear risk.

Benign hepatic adenomas should be considered in estrogen users having abdominal pain and tenderness, abdominal mass, or hypovolemic shock. Hepatocellular carcinoma has been reported in women taking estrogen-containing oral contraceptives.<sup>35</sup> Increased blood pressure may occur with use of estrogens in the menopause<sup>37</sup> and blood pressure should be monitored with estrogen use. A worsening of glucose tolerance has been observed in patients on estrogen-containing oral contraceptives. For this reason, diabetic patients should be carefully observed. Estrogens may lead to severe hypercalcemia in patients with breast cancer and bone metastases.

**PRECAUTIONS:** Physical examination and a complete medical and family history should be taken prior to the initiation of any estrogen therapy with special reference to blood pressure, breasts, abdomen, and pelvic organs, and should include a Papanicolaou smear. As a general rule, estrogen should not be prescribed for longer than one year without another physical examination being performed. Conditions influenced by fluid retention such as asthma, epilepsy, migraine, and cardiac or renal dysfunction, require careful observation. Certain patients may develop manifestations of excessive estrogenic stimulation, such as abnormal or excessive uterine bleeding, mastodynia, etc. Oral contraceptives appear to be associated with an increased incidence of mental depression.<sup>38,39</sup> Patients with a history of depression should be carefully observed. Preexisting uterine leiomyomata may increase in size during estrogen use. The pathologist should be advised of estrogen therapy when relevant specimens are submitted. If jaundice develops in any patient receiving estrogen, the medication should be discontinued while the cause is investigated. Estrogens should be used with care in patients with impaired liver function, renal insufficiency, metabolic bone diseases associated with hypercalcemia, or in young patients in whom bone growth is not complete.

The following changes may be expected with larger doses of estrogen:

- a. Increased sulfobromophthalein retention.
- b. Increased prothrombin and factors VII, VIII, IX, and X; decreased antithrombin 3; increased norepinephrine-induced platelet aggregability.
- c. Increased thyroid binding globulin (TBG) leading to increased circulating total thyroid hormone, as measured by PBI, T<sub>4</sub> by column, or T<sub>4</sub> by radioimmunoassay. Free T<sub>3</sub> resin uptake is decreased, reflecting the elevated TBG; free T<sub>4</sub> concentration is unaltered.
- d. Impaired glucose tolerance.
- e. Decreased pregnanediol excretion.
- f. Reduced response to methylprednisolone test.
- g. Reduced serum folate concentration.
- h. Increased serum triglyceride and phospholipid concentration.

As a general principle, the administration of any drug to nursing mothers should be done only when clearly necessary since many drugs are excreted in human milk.

**ADVERSE REACTIONS:** The following have been reported with estrogenic therapy, including oral contraceptives: breakthrough bleeding, spotting, change in menstrual flow; dysmenorrhea; premenstrual-like syndrome; amenorrhea during and after treatment; increase in size of uterine fibromyomata; vaginal candidiasis; change in cervical erosion and in degree of cervical secretion; cystitis-like syndrome; tenderness, enlargement, secretion (of breasts); nausea, vomiting, abdominal cramps, bloating; cholestatic jaundice; chloasma or melasma which may persist when drug is discontinued; erythema multiforme; erythema nodosum; hemorrhagic eruption; loss of scalp hair, hirsutism; steepening of corneal curvature; intolerance to contact lenses; headache, migraine, dizziness, mental depression, chorea; increase or decrease in weight; reduced carbohydrate tolerance; aggravation of porphyria; edema; changes in libido.

**ACUTE OVERDOSAGE:** May cause nausea, and withdrawal bleeding may occur in females.

**DOSAGE AND ADMINISTRATION:** 1. Given cyclically for short term use only. For treatment of moderate to severe vasomotor symptoms, atrophic vaginitis, or kraurosis vulvae associated with the menopause (0.3 to 1.25 mg or more daily).

The lowest dose that will control symptoms should be chosen and medication should be discontinued as promptly as possible.

Administration should be cyclic (e.g. three weeks on and one week off).

Attempts to discontinue or taper medication should be made at three to six month intervals.

2. Given cyclically: Female hypogonadism: Female castration. Primary ovarian failure. Osteoporosis.

Female hypogonadism—2.5 to 7.5 mg daily, in divided doses for 20 days, followed by a rest period of 10 days' duration. If bleeding does not occur by the end of this period, the same dosage schedule is repeated. The number of courses of estrogen therapy necessary to produce bleeding may vary depending on the responsiveness of the endometrium.

If bleeding occurs before the end of the 10 day period, begin a 20 day estrogen-progestin cyclic regimen with PREMARIN (Conjugated Estrogens Tablets, U.S.P.), 2.5 to 7.5 mg daily in divided doses, for 20 days. During the last five days of estrogen therapy, give an oral progestin. If bleeding occurs before this regimen is concluded, therapy is discontinued and may be resumed on the fifth day of bleeding.

Female castration and primary ovarian failure—1.25 mg daily, cyclically. Adjust upward or downward according to response of the patient. For maintenance, adjust dosage to lowest level that will provide effective control.

Osteoporosis (to retard progression)—1.25 mg daily, cyclically.

3. Given for a few days: Prevention of postpartum breast engorgement—3.75 mg every four hours for five doses, or 125 mg every four hours for five days.

4. Given chronically: Inoperable progressing prostatic cancer—1.25 to 2.5 mg three times daily.

Inoperable progressing breast cancer in appropriately selected men and postmenopausal women—10 mg three times daily for a period of at least three months.

Patients with an intact uterus should be monitored for signs of endometrial cancer and appropriate measures taken to rule out malignancy in the event of persistent or recurring abnormal vaginal bleeding.

**HOW SUPPLIED:** PREMARIN (Conjugated Estrogens Tablets, U.S.P.) No. 865—Each purple tablet contains 2.5 mg in bottles of 100 and 1,000. No. 866—Each yellow tablet contains 1.25 mg in bottles of 100 and 1,000. Also in unit dose package of 100. No. 867—Each red tablet contains 0.625 mg in bottles of 100 and 1,000. Also in unit dose package of 100. No. 868—Each green tablet contains 0.3 mg in bottles of 100 and 1,000.

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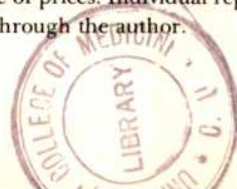
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is to 50 mcg

**OR OVER  
FROM  
ANOTHER  
50 mcg**

Your patient breaks through on her 50 mcg pill, and you don't want to increase her dose. Why not move her to 50 mcg OVRAL? In clinical studies, breakthrough bleeding occurred in only 1% of total cycles, spotting in 1.5% and amenorrhea in 0.7%. And the incidences of other minor side effects, such as nausea and vomiting, headache and weight gain, were also very low.\*

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## IN BRIEF:

**Indications and Usage**—OVRAL® is indicated for the prevention of pregnancy in women who elect to use oral contraceptives (OCs) as a method of contraception.

**Contraindications**—OCs should not be used in women with any of the following conditions: 1. Thrombophlebitis or thromboembolic disorders. 2. A past history of deep-vein thrombophlebitis or thromboembolic disorders. 3. Cerebral-vascular or coronary-artery disease. 4. Known or suspected carcinoma of the breast. 5. Known or suspected estrogen-dependent neoplasia. 6. Undiagnosed abnormal genital bleeding. 7. Known or suspected pregnancy (see Warning No. 5).

## Warnings

**Cigarette smoking increases the risk of serious cardiovascular side effects from oral contraceptive use. This risk increases with age and with heavy smoking (15 or more cigarettes per day) and is quite marked in women over 35 years of age. Women who use oral contraceptives should be strongly advised not to smoke.**

The use of oral contraceptives is associated with increased risk of several serious conditions, including thromboembolism, stroke, myocardial infarction, hepatic adenoma, gallbladder disease, hypertension. Practitioners prescribing oral contraceptives should be familiar with the following information relating to these risks.

1. **Thromboembolic Disorders and Other Vascular Problems:** An increased risk of thromboembolic and thrombotic disease associated with use of OCs is well established. Three principal studies in Great Britain and 3 in the U.S. have demonstrated increased risk of fatal and nonfatal venous thromboembolism and stroke, both hemorrhagic and thrombotic. These studies estimate that users of OCs are 4 to 11 times more likely than nonusers to develop these diseases without evident cause.

### CEREBROVASCULAR DISORDERS

In a collaborative American study of cerebrovascular disorders in women with and without predisposing causes, it was estimated that the risk of hemorrhagic stroke was 2.0 times greater in users than nonusers and the risk of thrombotic stroke was 4 to 9.5 times greater in users than in nonusers.

### MYOCARDIAL INFARCTION (MI)

An increased risk of MI associated with use of OCs has been reported, confirming a previously suspected association. These studies, conducted in the UK, found, as expected, that the greater the number of underlying risk factors for coronary-artery disease (cigarette smoking, hypertension, hypercholesterolemia, obesity, diabetes, history of preclampsia/tokemia), the higher the risk of developing MI, regardless of whether the patient was an OC user or not. OCs, however, were found to be a clear additional risk factor. In terms of relative risk, it has been estimated that OC users who do not smoke (smoking is considered a major predisposing condition to MI) are about twice as likely to have a fatal MI as nonusers who do not smoke. OC users who are also smokers have about a 5-fold increased risk of fatal MI compared to smokers who do not smoke, but about a 10- to 12-fold increased risk compared to nonusers who do not smoke. Furthermore, amount of smoking is also an important factor. In determining importance of these relative risks, however, baseline rates for various age groups must be given serious consideration. Importance of other predisposing conditions mentioned above in determining relative and absolute risks has not as yet been quantified, quite likely the same synergistic action exists, but perhaps to a lesser extent.

### RISK OF DOSE

In an analysis of data derived from several national adverse-reaction reporting systems, British investigators concluded that risk of thromboembolism, including coronary thrombosis, is directly related to dose of estrogen in OCs. Preparations containing 100 mcg or more of estrogen were associated with higher risk of thromboembolism than those containing 50-80 mcg. Their analysis did suggest, however, that quantity of estrogen may not be the sole factor involved. This finding has been confirmed in the U.S.

### ESTIMATE OF EXCESS MORTALITY FROM CIRCULATORY DISEASES

A large prospective study carried out in the UK estimated the mortality rate per 100,000 women per year from diseases of the circulatory system for users and nonusers of OCs according to age, smoking habits, and duration of use. Overall excess death rate annually from circulatory diseases for OC users was estimated to be 20 per 100,000 (ages 15-34—5/100,000; ages 35-44—33/100,000; ages 45-49—140/100,000), risk being concentrated in older women, in those with long duration of use, and in cigarette smokers. It was not possible, however, to examine interrelationships of age, smoking, and duration of use, nor to compare effects of continuous vs. intermittent use. Although the study showed a 10-fold increase in death due to circulatory diseases in users for 5 or more years, all these deaths occurred in women 35 or older. Until larger numbers of women under 35 with continuous use for 5 or more years are available, it is not possible to assess magnitude of relative risk for this younger group. Available data from a variety of sources have been analyzed to estimate risk of death associated with various methods of contraception. Estimates of risk of death for each method include combined risk of contraceptive method (e.g., thromboembolic and thrombotic disease in the case of OCs) plus risk attributable to pregnancy or abortion in event of method failure. This latter risk varies with effectiveness of method. The study concluded that mortality associated with all methods of birth control is low and below that associated with childbirth, with the exception of OCs in women over 40 who smoke. Lowest mortality is associated with condom or diaphragm backed up by early abortion. Risk of thromboembolic and thrombotic disease associated with OCs increases with age after about 30 and, for MI, is further increased by hypertension, hypercholesterolemia, obesity, diabetes, or history of preclampsia/tokemia, and especially cigarette smoking. Physician and patient should be alert to earliest manifestations of thromboembolic and thrombotic disorders (e.g., thrombophlebitis, pulmonary embolism, cerebrovascular insufficiency, coronary occlusion, retinal thrombosis, and mesenteric thrombosis). Should any of these occur or be suspected, the drug should be discontinued immediately. A 4- to 6-fold increased risk of postsurgery thromboembolic complications has been reported in OC users.

If feasible, OCs should be discontinued at least 4 weeks before surgery of a type associated with increased risk of thromboembolism or prolonged immobilization.

2. **Ocular Lesions:** There have been reports of neuro-ocular lesions such as optic neuritis or retinal thrombosis associated with use of OCs. Discontinue OCs if there is unexplained, sudden or gradual, partial or complete loss of vision; onset of proptosis or diplopia; papilledema, or retinal-vascular lesions, and institute appropriate diagnostic and therapeutic measures.

3. **Carcinoma:** Long-term continuous administration of either natural or

synthetic estrogen in certain animal species increases frequency of carcinoma of the breast, cervix, vagina, and liver. Certain synthetic progestogens, none currently contained in OCs, have been noted to increase incidence of mammary nodules, benign and malignant, in dogs. In humans, 3 case-control studies have reported an increased risk of endometrial carcinoma associated with prolonged use of exogenous estrogen in postmenopausal women. One publication reported on the first 21 cases submitted by physicians to a registry of cases of adenocarcinoma of the endometrium in women under 40 on OCs. Of cases found in women without predisposing risk factors (e.g., irregular bleeding at the time OCs were first given, polycystic ovaries), nearly all occurred in women who had used a sequential OC. These are no longer marketed. No evidence has been reported suggesting increased risk of endometrial cancer in users of conventional combination or progestogen-only OCs. Several studies have found no increase in breast cancer in women taking OCs or estrogens. One study, however, while also noting no overall increased risk of breast cancer in women on OCs, found an excess risk in subgroups of OC users with documented benign breast disease. Reduced occurrence of benign breast tumors in users of OCs has been well documented. In summary, there is at present no confirmed evidence from human studies of increased risk of cancer associated with OCs. Close clinical surveillance of all women on OCs is, nevertheless, essential. In all cases of undiagnosed persistent or recurrent abnormal vaginal bleeding, appropriate diagnostic measures should be taken to rule out malignancy. Women with a strong family history of breast cancer or with breast nodules, fibrocystic disease, or abnormal mammograms should be monitored with particular care if they elect to use OCs.

4. **Hepatic Tumors:** Benign hepatic adenomas have been found to be associated with use of OCs. One study showed that OCs with high hormonal potency were associated with higher risk than lower potency OCs. Although benign, hepatic adenomas may rupture and may cause death through intra-abdominal hemorrhage. This has been reported in short-term as well as long-term users. Two studies relate risk with duration of use of OCs, the risk being much greater after 4 or more years' use. While hepatic adenoma is rare, it should be considered in women presenting abdominal pain and tenderness, abdominal mass or shock. A few cases of hepatocellular carcinoma have been reported in women on OCs. Relationship of these drugs to this type of malignancy is not known.

5. **Use in or Immediately Preceding Pregnancy: Birth Defects in Offspring and Malignancy in Female Offspring.** Use of female sex hormones—both estrogenic and progestational agents—during early pregnancy may seriously damage the offspring. It has been shown that females exposed in utero to diethylstilbestrol, a nonsteroidal estrogen, have increased risk of developing in later life a form of vaginal or cervical cancer ordinarily extremely rare. This risk has been estimated to be of the order of 1 in 1,000 exposures or less. Although there is no evidence now that OCs further enhance risk of developing this type of malignancy, such patients should be monitored with particular care if they elect to use OCs. Furthermore, 30 to 90% of such exposed women have been found to have epithelial changes of the vagina and cervix. Although these changes are histologically benign, it is not known whether this condition is a precursor of vaginal malignancy. Male children so exposed may develop abnormalities of the urogenital tract. Although similar data are not available with use of other estrogens, it cannot be presumed they would not induce similar changes. An increased risk of congenital anomalies, including heart defects and limb defects, has been reported with use of sex hormones, including OCs, in pregnancy. One case-control study estimated a 4.7-fold increase in risk of limb-reduction defects in infants exposed in utero to sex hormones (OCs, hormonal withdrawal tests for pregnancy or attempted treatment for threatened abortion). Some exposures involved only a few days. Data suggest that risk of limb-reduction defects in exposed fetuses is somewhat less than 1 in 1,000 live births. In the past, female sex hormones have been used during pregnancy in an attempt to treat threatened or habitual abortion. There is considerable evidence that estrogens are ineffective for these indications, and there is no evidence from well-controlled studies that progestogens are effective. There is some evidence that triploidy and possibly other types of polyplidy are increased among abortuses from women who become pregnant soon after ceasing OCs. Embryos with these anomalies are virtually always aborted spontaneously. Whether there is an overall increase in spontaneous abortion of pregnancies conceived soon after stopping OCs is unknown. It is recommended that, for any patient who has missed 2 consecutive periods, pregnancy should be ruled out before continuing OCs. If the patient has not adhered to the prescribed schedule, the possibility of pregnancy should be considered at time of first missed period, and further use of OCs should be withheld until pregnancy has been ruled out. If pregnancy is confirmed, the patient should be apprised of the potential risks to the fetus, and advisability of continuation of the pregnancy should be discussed. It is also recommended that women who discontinue OCs with intent of becoming pregnant use an alternate form of contraception for a period of time before attempting to conceive. Many clinicians recommend 3 months, although no precise information is available on which to base this. The administration of progestogen-estrogen combinations to induce withdrawal bleeding should not be used as a test of pregnancy.

6. **Gallbladder Disease:** Studies report increased risk of surgically confirmed gallbladder disease in users of OCs and estrogens. In one study, increased risk appeared after 2 years' use and doubled after 4 or 5 years' use. In one of the other studies, increased risk was apparent between 6 and 12 months' use.

7. **Carbohydrate and Lipid Metabolic Effects:** Decrease in glucose tolerance has been observed in a significant percentage of patients on OCs. For this reason, prediabetic and diabetic patients should be carefully observed while on OCs. Increase in triglycerides and total phospholipids has been observed in patients on OCs, clinical significance of this finding remains to be defined.

8. **Elevated Blood Pressure:** Increase in blood pressure has been reported in patients on OCs. In some women, hypertension may occur within a few months of beginning OCs. In the 1st year of use, prevalence of women with hypertension is low in users and may be no higher than that of a comparable group of nonusers. Prevalence in users increases, however, with longer exposure, and in the 5th year of use is 2 1/2 to 3 times the reported prevalence in the 1st year. Age is also strongly correlated with development of hypertension in OC users. Women who previously have had hypertension during pregnancy may be more likely to develop elevation of blood pressure on OCs. Hypertension that develops as a result of taking OCs usually returns to normal after discontinuing the drug.

9. **Headache:** Onset or exacerbation of migraine or development of headache of a new pattern which is recurrent, persistent, or severe, requires discontinuation of OCs and evaluation of the cause.

10. **Bleeding Irregularities:** Breakthrough bleeding, spotting, and amenorrhea are frequent reasons for patients discontinuing OCs. In breakthrough bleeding, as in all cases of irregular vaginal bleeding,

nonfunctional causes should be borne in mind. In undiagnosed persistent or recurrent abnormal bleeding from the vagina, adequate diagnostic measures are indicated to rule out pregnancy or malignancy. If pathology has been excluded, time or change to another OC may solve the problem. Changing to an OC with a higher estrogen content, while potentially useful in minimizing menstrual irregularity, should be done only if necessary, since this may increase risk of thromboembolic disease. Women with past history of oligomenorrhea or secondary amenorrhea or young women without regular cycles may have a tendency to remain anovulatory or to become amenorrheic after discontinuing OCs. Women with these preexisting problems should be advised of this possibility and encouraged to use other methods. Post-use anovulation, possibly prolonged, may also occur in women without previous irregularities.

11. **Ectopic Pregnancy:** Ectopic as well as intrauterine pregnancy may occur in contraceptive failures.

12. **Breast-feeding:** OCs given in the postpartum period may interfere with lactation and decrease quantity and quality of breast milk. Furthermore, a small fraction of the hormones in OCs has been identified in the milk of mothers on OCs; effects, if any, on the breast-fed child have not been determined. If feasible, defer OCs until infant has been weaned.

## Precautions—GENERAL

1. A complete medical and family history should be taken prior to initiation of OCs. Pretreatment and periodic physical examinations should include special reference to blood pressure, breasts, abdomen and pelvic organs, including Pap smear and relevant laboratory tests. As a general rule OCs should not be prescribed for longer than 1 year without another physical examination.

2. Under influence of estrogen-progestogen preparations, preexisting uterine leiomyomata may increase in size.

3. Patients with history of psychic depression should be carefully observed and the drug discontinued if depression recurs to a serious degree. Patients becoming significantly depressed while on OCs should stop OCs and use an alternate method to try to determine whether the symptom is drug-related.

4. OCs may cause some degree of fluid retention. They should be prescribed with caution, and only with careful monitoring, in patients with conditions which might be aggravated by fluid retention, such as convulsive disorders, migraine syndrome, asthma, or cardiac or renal insufficiency.

5. Patients with a past history of jaundice during pregnancy have an increased risk of recurrence while on OCs. If jaundice develops, OCs should be discontinued.

6. Steroid hormones may be poorly metabolized in patients with impaired liver function and should be administered with caution.

7. OC users may have disturbances in normal tryptophan metabolism which may result in a relative pyridoxine deficiency. Clinical significance is undetermined.

8. Serum folate levels may be depressed by OCs. Since the pregnant woman is predisposed to development of folate deficiency and incidence of folate deficiency increases with increasing gestation, it is possible that if a woman becomes pregnant shortly after stopping OCs, she may have a greater chance of developing folate deficiency and complications attributed to this deficiency.

9. The pathologist should be advised of OC therapy when relevant specimens are submitted.

10. Certain endocrine- and liver-function tests and blood components may be affected by estrogen-containing OCs:

a. Increased sulfobromophthalen retention; b. Increased prothrombin and factors VII, VIII, IX, and X; decreased antithrombin 3; increased norepinephrine-induced platelet aggregability; c. Increased thyroid-binding globulin (TBG) leading to increased circulating total-thyroid hormone, as measured by protein-bound iodine (PBI), T4 by column, or T4 by radioimmunoassay. Free T3 resin uptake is decreased, reflecting the elevated TBG; free T4 concentration is unaltered; d. Decreased pregnandiol excretion; e. Reduced response to methylpyruvate test.

## Information for the Patient—See Patient Package Labeling

**Drug Interactions**—Reduced efficacy and increased incidence of breakthrough bleeding have been associated with concomitant use of rifampin. A similar association has been suggested with barbiturates, phenylbutazone, phenytoin sodium, and ampicillin.

**Carcinogenesis**—See Warnings on carcinogenic potential of OCs.

**Pregnancy—Category X.** See Contraindications, Warnings.

**Nursing Mothers**—See Warnings.

**Adverse Reactions**—An increased risk of these serious adverse reactions has been associated with use of OCs (see Warnings). Thrombophlebitis, pulmonary embolism, coronary thrombosis, cerebral thrombosis, cerebral hemorrhage, hypertension, gallbladder disease, benign hepatomas, congenital anomalies. There is evidence of an association between the following conditions and use of OCs although additional confirmatory studies are needed: mesenteric thrombosis, neuro-ocular lesions, e.g., retinal thrombosis and optic neuritis.

The following adverse reactions have been reported in patients on OCs and are believed to be drug-related. Nausea and/or vomiting, usually the most common adverse reactions, occur in approximately 10 percent or less of patients during the first cycle. Other reactions, as a general rule, are seen much less frequently or only occasionally. Gastrointestinal symptoms (such as abdominal cramps and bloating), breakthrough bleeding, spotting, change in menstrual flow, dysmenorrhea, amenorrhea during and after treatment, temporary infertility after discontinuance of treatment, edema, chloasma or melasma which may persist, breast changes: tenderness, enlargement, and secretion, change in weight (increase or decrease), change in cervical erosion and cervical secretion, possible diminution in lactation when given immediately postpartum; cholestatic jaundice; migraine, increase in size of uterine leiomyomata; rash (allergic), mental depression, reduced tolerance to carbohydrates, vaginal candidiasis, change in corneal curvature (steepening), intolerance to contact lenses.

The following adverse reactions have been reported in users of OCs, and the association has been neither confirmed nor refuted: premenstrual-like syndrome, cataracts, changes in libido, chorea, changes in appetite, cystitis-like syndrome, headache, nervousness, dizziness, hirsutism, loss of scalp hair, erythema multiforme, erythema nodosum, hemorrhagic eruption, vaginitis, porphyria.

**Acute Overdose**—Serious ill effects have not been reported following acute ingestion of large doses of OCs by young children. Overdose may cause nausea, and withdrawal bleeding may occur in females.

**Ovral** each tablet contains 0.5 mg norgestrel with 0.05 mg ethinyl estradiol

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Transactions of the Thirty-sixth  
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of Obstetricians and  
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Significance of intrauterine adhesions detected  
hysteroscopically in eumenorrheic infertile women  
and role of antecedent curettage in their formation

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The increasing use of hysteroscopy as a diagnostic tool in the infertile woman has shown that intrauterine adhesions are of more concern, especially in eumenorrheic women, than previously was recognized. Their significance is not yet clear. This study was designed to compare the incidence of such lesions detected hysteroscopically in 235 women with otherwise unexplained infertility with their incidence in a group of 31 potentially fertile women. The outcome after treatment was recorded. The effect of curettage in the production of these adhesions was examined. The incidence of adhesions in the infertile group was considerably greater than in the potentially fertile group ( $p < 0.05$ ). A 50% pregnancy rate was achieved by lysis of adhesions. An antecedent history of curettage was associated with a significantly higher incidence of adhesion formation than that found in noncuretted patients ( $p < 0.0005$ ). (AM. J. OBSTET. GYNECOL. 139:239, 1981.)

ASHERMAN'S SYNDROME of amenorrhoea traumaticum was characterized by amenorrhea, intrauterine adhesions, and infertility.<sup>1</sup> Curettage, either postabortion or postpartum, appeared to be the most important

event in the formation of the adhesions. Subsequent reports<sup>2, 3</sup> have shown that intrauterine adhesions do not invariably produce amenorrhea. Until recently hysterosalpingography has been used to diagnose such lesions, and their removal has been demonstrated to improve reproductive performance.<sup>4</sup> Filmy intrauterine adhesions have been noted in regularly menstruating patients undergoing hysteroscopy as part of the investigation of infertility.<sup>5-7</sup>

This study was designed to determine if the incidence of filmy intrauterine adhesions in an infertile eumenorrheic population differed from that in an apparently fertile eumenorrheic population. Evidence was sought to determine whether removal of such lesions would enhance reproductive performance. The relationship between a history of earlier curettage and

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**Table I.** Relevant clinical data with respect to 266 patients examined hysteroscopically

|                                 | <i>Infertility</i> | <i>Previous<br/>tubal ligation</i> |
|---------------------------------|--------------------|------------------------------------|
| Total No. of patients           | 235                | 31                                 |
| Ages (years)                    | 18-35              | 26-37                              |
| Duration of infertility (years) | 1-11               | —                                  |
| Primary infertility             | 129                | —                                  |
| Secondary infertility           | 106                | 31                                 |

the subsequent hysteroscopic detection of intrauterine adhesions was examined.

### Patients and methods

Patients who attended the University of Calgary Endocrine/Infertility Clinic underwent a full investigation which included a history and physical examination and detection of presumptive evidence of ovulation by basal body temperature graphing and, where indicated, by serum progesterone assays or timed endometrial biopsy.

Two semen analyses were performed on each male partner after a history and complete physical examination had been recorded. A postcoital test was carried out at the appropriate time in the second apparently ovulatory cycle. Only those patients in whom no abnormalities were detected were considered acceptable for the study. All were asked detailed and specific questions with respect to their menstrual history. Two hundred sixty-six patients had not experienced any change in their menstrual cycles. These women were divided into two groups. The first group comprised 235 women with unexplained infertility. The second group comprised 31 women of previously proved fertility whose only present bar to conception was a recently performed tubal sterilization. The clinical data with respect to these 266 women are shown in Table I. Specific details about the patients' antecedent histories were collected. Particular attention was paid to the finding of a history of dilatation and curettage, pelvic inflammatory disease, uterine surgery, abortion (spontaneous or therapeutic), cesarean section, and postpartum or postabortion sepsis.

Any of these occurrences were considered noteworthy if detected in patients complaining of primary infertility. In those patients who previously had been sterilized or who were complaining of secondary infertility, only those historical factors which had occurred during or following the last pregnancy were accorded any significance.

Particular attention was paid to a history of previous curettage and it was determined whether this event had

**Table II.** Reasons for failure of hysteroscopy in 266 patients

| <i>Reason for failure</i> | <i>No. of cases N = 266)</i> |
|---------------------------|------------------------------|
| Failure to dilate cervix  | 6                            |
| Air bubbles in media      | 3                            |
| Obesity                   | 1                            |
| Intrauterine pregnancy    | 1                            |
| Total failures            | 11 (4.1%)                    |

been diagnostic or to complete either a spontaneous or therapeutic abortion.

All patients underwent combined laparoscopy and hysteroscopy under general anesthesia as previously described.<sup>7</sup> The hysteroscopy was performed with Hyskon\* (dextran 70) used as the uterine distention medium.

The endoscopic findings were recorded on a standard form. For a diagnosis of intrauterine adhesions to be made, Sugimoto's<sup>8</sup> criteria had to be satisfied, that is, the adhesions had to be attached to the uterine walls at two or more points ("central adhesions") or form shelflike projections ("marginal adhesions"). It was not possible to obtain sufficient tissue for histologic confirmation in all cases and thus the diagnosis was made by hysteroscopic visualization only. When adhesions were noted they were removed either by pushing, against them with the tip of the hysteroscope or by very gentle curettage and subsequent hysteroscopic re-visualization to ensure removal. No patients required abdominal synechotomy and none was treated by any postoperative ancillary measures.

Those patients in whom the only finding was intrauterine adhesions were followed up to determine their reproductive outcomes subsequent to lysis of adhesions.

### Results

Hysteroscopy was performed successfully in 224 of 235 infertile patients and in all 31 of those who previously had been sterilized. The reasons for hysteroscopic failure are shown in Table II. The one serious complication, inadvertent visualization of an intrauterine pregnancy, has been reported elsewhere.<sup>7</sup> Eight of these failures occurred in patients with primary infertility and three were in patients with secondary infertility. The total failure rate in 266 patients was 4.1%. The lesions which were detected within the uterine cavity are shown in Table III. Of a total of 224 uterine cavities visualized in the infertile group, 134

\*Pharmacia, Piscataway, New Jersey.



were judged to be normal (59.8%) and 90 were judged to be abnormal (40.2%). Intrauterine adhesions were noted in only one of the 31 previously sterilized patients (3.2%) and in 48 infertile patients (21.4%) ( $p < 0.05$ ).

None of the previously sterilized patients and 76 of the 224 infertile patients had undergone earlier curettage. Thirty-seven of these curetted patients had adhesion formation, and 11 of the noncuretted patients had such lesions ( $p < 0.0005$ ). Of these 11 women two had undergone cesarean section, one had had postpartum sepsis, and four had a history suggestive of pelvic inflammatory disease. Four patients, including one with primary infertility, showed no evidence of antecedent events which might have been causative.

Table IV provides details with respect to the nature of the curettage performed in patients with both primary and secondary infertility.

Of the 48 infertile patients with intrauterine adhesions 15 had no other demonstrable cause of infertility. The remaining 33 had tubal or peritubal disease (detected laparoscopically) of sufficient severity to be judged causative.

The subsequent reproductive outcomes of these 15 patients are shown in Table V.

#### Comment

Arguments and data to support replacing hysterosalpingography by combined laparoscopy and hysteroscopy as the initial investigation of women with otherwise unexplained infertility have been addressed elsewhere.<sup>5-7, 9</sup> In this series the failure rate of hysteroscopy was 4.1% (Table II). The one complication attributed to the procedure, the inadvertent visualization of an intrauterine pregnancy, was, in retrospect, avoidable. Details of the case have been reported.<sup>7</sup>

It would appear that hysteroscopy is a relatively simple and safe procedure. Whether or not it is of value in the investigation of the infertile woman will depend upon the significance of such lesions as polyps and adhesions which are detected hysteroscopically. The overall detection rate of intrauterine abnormality of 41.2%, although apparently high, is in keeping with a 45.5% rate in 528 patients reported in the world literature.<sup>10</sup>

Although for completeness all positive hysteroscopic findings are mentioned in this article (Table III), we have tried to define the significance of filmy intrauterine adhesions only.

That dense intrauterine adhesions, associated with amenorrhea or hypomenorrhea, can cause infertility is not in doubt.<sup>1-3</sup> That filmy adhesions may exist within the uterus of a regularly menstruating woman com-

**Table III.** Findings at hysteroscopy in 255 patients

| Uterine cavity findings<br>(N = 255) | Primary infertility<br>(n = 121) | Secondary infertility<br>(n = 103) | Previous sterilization<br>(n = 31) |
|--------------------------------------|----------------------------------|------------------------------------|------------------------------------|
| Normal                               | 87 (71.9%)                       | 47 (45.6%)                         | 30 (96.8%)                         |
| Polyps                               | 18 (14.8%)                       | 16 (15.5%)                         | 0                                  |
| Adhesions                            | 12 (9.9%)                        | 36 (34.9%)                         | 1 (3.2%)                           |
| Fibroids                             | 4 (3.3%)                         | 3 (2.9%)                           | 0                                  |
| Adhesions and polyps                 | 0                                | 1 (0.97%)                          | 0                                  |

**Table IV.** Nature of curettage performed compared with detection of intrauterine adhesions in 224 infertile patients

| Nature of curettage                            | Hysteroscopic findings (N = 224) |                           |
|------------------------------------------------|----------------------------------|---------------------------|
|                                                | Adhesions<br>(n = 48)            | No adhesions<br>(n = 176) |
| Primary infertility,<br>Diagnostic curettage   | 11                               | 15                        |
| Secondary infertility,<br>Diagnostic curettage | 18                               | 12                        |
| Abortion by curettage                          | 8                                | 12                        |
| Total No. of<br>patients curetted              | 37                               | 39                        |

**Table V.** Reproductive outcomes in 15 patients whose only apparent cause of infertility was intrauterine adhesions

| Outcome                            | No. of patients (N = 15) |
|------------------------------------|--------------------------|
| Lost to follow-up                  | 2                        |
| Followed up for less than 6 months | 1                        |
| First-trimester abortion           | 1                        |
| Pregnancy in midtrimester          | 1                        |
| Term delivery                      | 5                        |
| Not pregnant                       | 5                        |

plaining of infertility is a relatively recent finding. Sugimoto<sup>8</sup> noted that 72 of 192 patients in whom a hysteroscopic diagnosis of intrauterine adhesions had been made were eumenorrheic. The removal of these adhesions resulted in an improvement of reproductive performance. In our series such lesions were noted in 48 of 224 eumenorrheic infertile women (12.8%). Although no comment was made by either group as to the menstrual status of their patients, Lindemann and Mohr<sup>11</sup> reported an incidence of 11% and Sciarra and Valle<sup>12</sup> found adhesions in 22.2% of their infertile patients.

The occurrence of such adhesions in otherwise healthy women who have no difficulty conceiving is not known, nor are such data easily obtained. In order to gain partial insight into this problem it was assumed

that women requesting reversal of a previously performed tubal sterilization were otherwise fertile. In this group of 31 women only one had evidence of adhesions. When the occurrence of adhesions in both the infertile and previously sterilized group was compared (chi-square test,  $P < 0.05$ ) it was concluded that it is probable that such lesions do occur more frequently in an infertile population.

The fact that lesions are detected more frequently in an infertile population does not substantiate a cause-and-effect relationship. Correction of any apparent defect should result in improved reproductive performance. Only 15 of our patients had intrauterine adhesions as the only apparent cause of their infertility. Twelve of these patients have been followed up for longer than 6 months. Five patients have given birth to viable children and one is at 27 weeks' gestation (Table V). It must not be overlooked that up to 43% of patients may conceive within 6 months of a laparoscopy and perhydrotubation.<sup>13</sup> As the latter procedure was performed in addition to hysteroscopy in all 15 patients it is possible that an apparent therapeutic benefit derived from lysis of these adhesions was a function of the perhydrotubation. Sugimoto<sup>8</sup> has reported a larger series and a pregnancy rate of 41.2% was achieved in 192 patients in whom lysis of intrauterine adhesions was performed.

In our series 37 of 48 women with adhesions had undergone curettage. Twenty-nine of these procedures were for diagnostic purposes and eight had been to complete either a spontaneous or a therapeutic abortion (Table IV). Of the remaining 11 patients only four had no antecedent history of either infection or intrauterine operations. When the incidence of adhesion formation in the 76 curetted patients was compared with the incidence in the 148 noncuretted patients (chi-square test,  $P < 0.0005$ ), a highly significant relationship appeared. Previous authors<sup>1-3</sup> have drawn attention to the relationship between postabortion or

postpartum curettage and Asherman's syndrome. Sugimoto<sup>8</sup> postulated that this is due to the greater ease with which the basal layer is removed from the soft, pregnant uterus. In his series, only one patient, who was hypomenorrheic, had undergone diagnostic curettage. The finding of adhesions in eight of 20 women who had curettage to complete abortion is not surprising.

That 29 of a total of 56 women who had undergone a diagnostic curettage should have evidence of such adhesions and should account for 60.4% of the total group of women who had adhesions demonstrated is of greater interest. When it is considered that all our patients were eumenorrheic and that all the adhesions were not dense, it is not unreasonable to suggest that in the nonpregnant state simple diagnostic curettage may denude lesser areas of the basal layer. This denudation would result in the formation of less dense adhesions than those seen in full-blown Asherman's syndrome and cause less disruption of menstrual function.

A valid criticism may be that the adhesions which are described in this report were not submitted to histologic scrutiny. In many cases recovery of tissue proved to be impossible, particularly when lysis was achieved by the method of pushing with the hysteroscope.<sup>8</sup> As we concur with Sugimoto<sup>8</sup> that "the components of intrauterine adhesions can be decided from hysteroscopic observation alone," visualization was used as the sole diagnostic criterion.

We conclude that a history of previous curettage (whether diagnostic or to complete an abortion) in a woman of otherwise unexplained infertility, even in the presence of eumenorrhea, should constitute an indication for hysteroscopy. Although the supporting data are less clear, comparison between infertile women and previously sterilized women would suggest that filmy intrauterine adhesions may play a role in the causation of infertility. Further studies are required to determine if removal of such lesions may result in improved reproductive performance.

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# A protein-binding radioassay for 6 $\beta$ -hydroxycortisol: Detection in pregnancy urine and amniotic fluid

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A major metabolic step in the excretion of cortisol during pregnancy is its hydroxylation to 6 $\beta$ -hydroxycortisol (6 $\beta$ -OHF), which is a prevalent unconjugated polar metabolite in maternal urine and amniotic fluid. The fetus may be a major contributor to the maternal pool of 6 $\beta$ -OHF. Objectives of this study were to establish levels of 6 $\beta$ -OHF before and during normal pregnancy. Until recently the lack of practical methods for routine measurement of 6 $\beta$ -OHF has limited the investigation of this polar metabolite as a potential diagnostic aid. A protein-binding radioassay for 6 $\beta$ -OHF has been developed which requires a preliminary extraction step and separation by column chromatography. Specificity, precision, and sensitivity of the method are reported. The mean normal 24-hour urine levels of 6 $\beta$ -OHF in male subjects (300  $\mu$ g), female subjects not receiving estrogenic medication (313  $\mu$ g), and female subjects using oral contraceptives (227  $\mu$ g) did not differ significantly. However, during pregnancy, urinary levels increased to 430  $\mu$ g during the first trimester and 863  $\mu$ g during the third trimester. Amniotic fluid levels of 6 $\beta$ -OHF increased from a mean of 5 ng/ml during the first trimester to 19 ng/ml during the third trimester. The present assay offers a reliable clinical laboratory method for the specific measurement of 6 $\beta$ -OHF in high-risk pregnancies. (AM. J. OBSTET. GYNECOL. 139:243, 1981.)

CONJUGATION AND HYDROXYLATION of cortisol may each serve the same purpose under different circumstances. The primary route of cortisol metabolism in the liver involves A-ring reduction<sup>1</sup> and subsequent conjugation with glucuronic acid.<sup>2</sup> An alternate disposal route has also been demonstrated in which cortisol is hydroxylated, primarily at carbon-6, whereby it is rendered more polar and thus capable of urinary excretion in an unconjugated state.<sup>3, 4</sup> The most abundant unconjugated corticoid in human urine is 6 $\beta$ -hydroxycortisol (6 $\beta$ -OHF) or 6 $\beta$ ,11 $\beta$ ,17 $\alpha$ ,21-tetrahy-

droxy-4-pregnene-3-20-dione. The existence of a 6-hydroxysteroid in human urine was first reported by Lieberman and associates<sup>5</sup> and identified by Burstein and associates<sup>6</sup> as 6 $\beta$ -OHF. Subsequent investigations reported 6 $\beta$ -OHF to be present in neonatal urine,<sup>4</sup> amniotic fluid,<sup>7</sup> placenta, adrenal gland, and liver, with the latter tissue displaying the highest degree of carbon-6 hydroxylation.<sup>8</sup>

Increased urinary excretion of 6 $\beta$ -OHF during pregnancy<sup>3</sup> has been attributed to the influence of estrogens. Evidence suggests that estrone<sup>8</sup> and diethylstilbestrol<sup>9</sup> may impair A-ring reduction of cortisol.<sup>10</sup> Other conditions such as adrenocortical hyperfunction,<sup>11, 12</sup> liver cirrhosis,<sup>13</sup> and certain drug treatments<sup>14, 15</sup> also manifest an elevated excretion of 6 $\beta$ -OHF. The metabolism of cortisol by hydroxylation was comprehensively reviewed by Cope.<sup>16</sup>

At the time this investigation was started, methods used to measure 6 $\beta$ -OHF required tedious paper chromatography steps and estimation by colorimetric analysis.<sup>3, 4, 17</sup> Clinical and experimental investigations of 6 $\beta$ -OHF have been limited by the lack of a practical method for routine measurement of this extremely polar metabolite. The objectives of the present investi-

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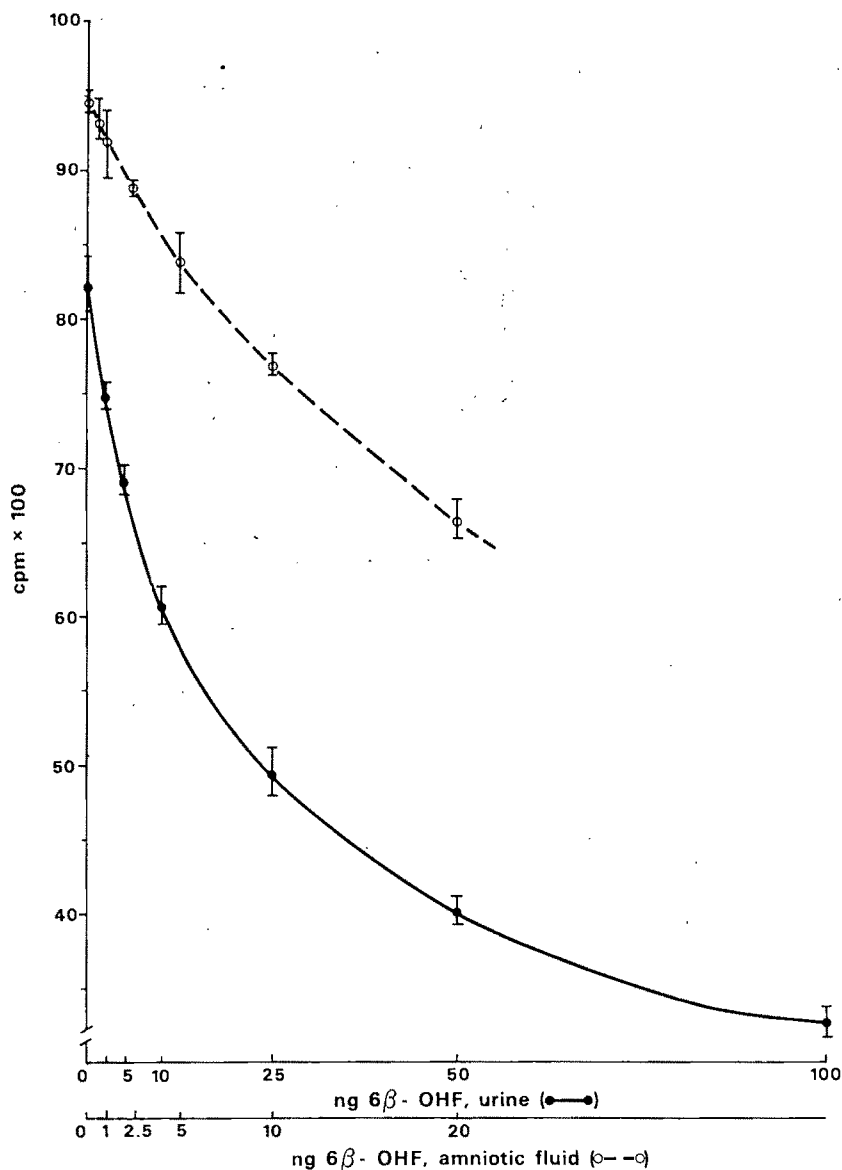


Fig. 1. Standard curves used for the radioassay of  $6\beta$ -OHF in urine and amniotic fluid. Each point represents the mean counts per minute bound to CBG and the range of values for triplicate determinations.

gation were to develop a protein-binding radioisotope assay of sufficient sensitivity to quantitate reliably levels of  $6\beta$ -OHF in maternal urine and amniotic fluid at various stages of gestation.

#### Material and methods

**Clinical material.** Urine was collected for 24 hours from groups of 9 to 18 normal subjects (see Fig. 3). Mean ages  $\pm$  SEM were as follows: male subjects,  $29 \pm 6$  years; female subjects receiving estrogenic medication (Ortho-Novum 1/50, 4; Norinyl 2; Minovral, 4),  $30 \pm 5$  years; female subjects not using oral contraceptive medication,  $32 \pm 4$  years; pregnant patients

in the first trimester (8 to 14 weeks),  $27 \pm 6$  years; and pregnant patients in the third trimester (30 to 41 weeks),  $28 \pm 5$  years. Amniotic fluid specimens (10 ml) were obtained by amniocentesis performed with the use of ultrasonography on patients admitted to Foothills Provincial General Hospital in Calgary for either prenatal screening or measurement of fetal maturity, and other specimens were obtained at the time of labor and delivery. All clinical samples were stored at  $-20^\circ\text{C}$ .

**Steroids.** All steroids were obtained from commercial sources (Steraloids Inc., Wilton, New Hampshire, and Sigma Chemical Co., St. Louis, Missouri) except for the gifts of  $2\alpha$ - and  $2\beta$ -hydroxycortisol. Steroids



**Table I.** Cross-reactivity of steroids with 6β-hydroxycortisol and their elution profile from an LH-20 Sephadex column

| Steroid                  | Cross-reactivity with<br>6β-OHF radio-receptor assay* | Fractions† from LH-20 column<br>containing 85% or more of steroid sample |
|--------------------------|-------------------------------------------------------|--------------------------------------------------------------------------|
| Progesterone             | 7.4                                                   | 2-3‡                                                                     |
| 20α-Dihydroprogesterone  | 4.1                                                   | 2-4‡                                                                     |
| 17α-Hydroxyprogesterone  | 4.1                                                   | 3-4‡                                                                     |
| Pregnanediol             | 0.4                                                   | 3§                                                                       |
| Pregnanetriol            | 0.05                                                  | 4§                                                                       |
| Estrone                  | 0.2                                                   | 5                                                                        |
| 17β-Estradiol            | 0.05                                                  | 6-8‡                                                                     |
| Estriol                  | 0.1                                                   | 12-14‡                                                                   |
| Estetrol                 | <0.05                                                 | 18-21                                                                    |
| Androsterone             | 1.2                                                   | 3§                                                                       |
| Etiocholanolone          | 1.0                                                   | 3§                                                                       |
| Testosterone             | 5.7                                                   | 3-4‡                                                                     |
| Corticosterone           | 4.8                                                   | 5-6‡                                                                     |
| Cortisone                | 9.8                                                   | 7-8                                                                      |
| Cortisol                 | 950.0                                                 | 8-9¶                                                                     |
| Substance X              | Positive                                              | 8-14¶                                                                    |
| Tetrahydrocorticosterone | <0.05                                                 | 6-8§                                                                     |
| Tetrahydrocortisone      | 0.3                                                   | 6-9¶                                                                     |
| Tetrahydrocortisol       | 0.1                                                   | 9-12¶                                                                    |
| 2α-Hydroxycortisol       | 9.5                                                   | 12-14¶                                                                   |
| 2β-Hydroxycortisol       | 6.8                                                   | 12-14¶                                                                   |
| 6β-Hydroxycortisone      | 0.4                                                   | 12-14§                                                                   |
| 6β-Hydroxycortisol       | 100.0                                                 | 16-20¶                                                                   |

\*The percentage of cross-reactivity was measured as  $\frac{\text{equivalent ng } 6\beta\text{-OHF}}{\text{ng steroid added to assay}} \times 100$ . (In all cases 1,000 ng of each steroid was used to measure cross-reactivity, except for cortisol where 1 ng was sufficient.)

†Each fraction = 300 drops (approximately 6 ml).

‡Position at which steroid was eluted from the LH-20 column was detected by isotopically labeled steroid.

§Position at which steroid was eluted from the LH-20 column was detected by presence of white residue in tubes after drying fractions.

||Position at which steroid was eluted from the LH-20 column was detected by ultraviolet absorbance.

¶Position at which steroid was eluted from the LH-20 column was detected by cross-reactivity in radioassay.

labeled isotopically were obtained from New England Nuclear, Boston, Massachusetts.

**Extraction of 6β-OHF from biological fluids.** To each 5 ml aliquot of urine or amniotic fluid was added 0.1 ml of recovery solution containing 25,000 cpm of 6β-hydroxycortisol-<sup>3</sup>H(G)/ml of methanol (specific activity 3.0 Ci/mmol). The samples were then extracted by a modification of the method of Frantz and associates.<sup>11</sup> Samples were extracted once with 5 ml of ethyl acetate for urine and 15 ml of ethyl acetate for amniotic fluid. After washing with sodium hydroxide, acetic acid, and water, the extracts were centrifuged to remove remaining water droplets and then evaporated to dryness at 40° C under nitrogen and the dried extracts were stored at 4° C.

**Separation of steroids by column chromatography.** Dried extracts were dissolved in 1 ml of the chromatography solvent system containing analytic grade hexane:benzene:methanol (65:15:20). Each sample was then applied to the top of a glass chromatography column (0.9 by 25 cm, Bio-Rad Laboratories, Richmond, California) packed to a height of 21 cm with

Sephadex LH-20, particle size graded according to the custom lot No. 96-39-5-A (Pharmacia, Sweden). The samples were eluted with the solvent system, and 6 ml fractions were collected. Under these conditions 6β-OHF was eluted in fractions Nos. 16 to 20 inclusive and, provided that the Sephadex was not allowed to dry, a constant elution profile (monitored with <sup>3</sup>H-6β-OHF) was obtained for several months without repacking the columns. Fractions 16 to 20 were pooled, evaporated to dryness at 40° C under nitrogen, and stored at 4° C.

**Recovery determination.** Recovery measurements were made for each sample to enable correction for losses during the extraction and separation procedures. Fractions 16 to 20 were dissolved in 1 ml of assay buffer (0.01M phosphate, pH 7.4, containing 1.15 gm of sodium phosphate, 0.258 gm of potassium phosphate, 4.09 gm of sodium chloride, 1.0 gm of bovine serum albumin fraction V, and 1.0 gm of sodium azide per liter). From each of these 1 ml test solutions 0.5 ml was counted on a scintillation counter for 10 minutes. The percentage recovery was then calculated by com-

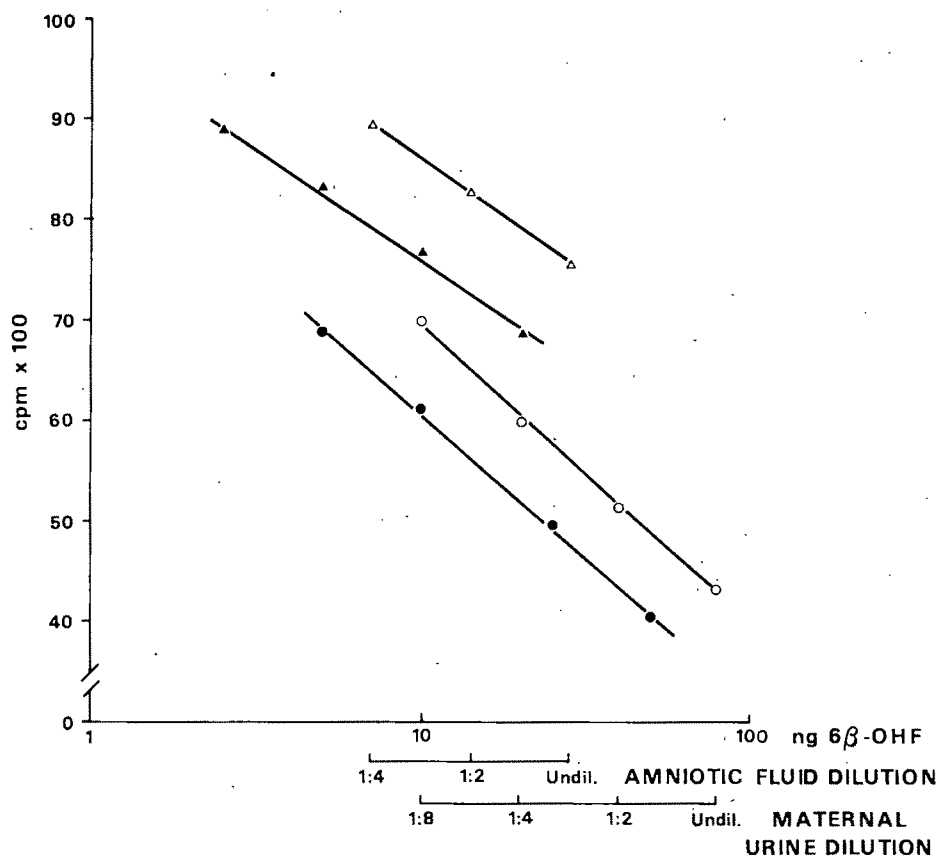


Fig. 2. Comparison of dose-response dilution curves of maternal urine (○) and amniotic fluid (Δ) containing 6β-OHF to curves obtained from pure 6β-OHF standard used to assay urine (●) and amniotic fluid (▲). Each point represents the mean of triplicate determinations.

paring the recovery counts from each test solution with a "100% recovery" vial containing 0.1 ml of the recovery solution added to each specimen before extraction (2,500 cpm of  $^3\text{H}$ -6β-OHF in methanol) and 0.5 ml of assay buffer. The addition of buffer was essential to ensure that this vial was subjected to the same quenching effect as the test vial was exposed to. Typical recoveries ranged from 28% to 52% in a group of 20 urine and 10 amniotic fluid specimens (mean of 47% ± 5%).

**Quantitation of 6β-OHF.** Levels of 6β-OHF were found to be different in urine and amniotic fluid; therefore, separate standard curves were constructed (Fig. 1). Dog plasma, "stripped" of steroids with 30 to 60 mesh Florisil (Matheson, Coleman & Bell, Norwood, Ohio), was used as the source of corticosteroid-binding globulin (CBG)<sup>18</sup> for this radioassay. The dog plasma was diluted with distilled water to 1% for the assay of urine samples and to 0.5% for amniotic fluid. Both solutions were labeled by the addition of  $^3\text{H}$ -cortisol to give approximately 12,000 cpm/ml and then incubated

at 4° C for at least 12 hours prior to use, the solutions being stable at this temperature for 2 months. Standard curves were constructed with the use of seven standard solutions ranging from 0 to 100 ng of 6β-OHF per 0.2 ml of assay buffer for the assay of urine and 0 to 20 ng of 6β-OHF per 0.2 ml for amniotic fluid.

The assay was carried out in triplicate in 10 by 75 mm glass tubes. For the standard curve 0.2 ml aliquots of each standard solution were added to assay tubes. From each of the test solutions (0.5 ml remaining after recovery determination) aliquots were added to assay tubes (0.025 ml for urine extracts, 0.1 ml for amniotic fluid) and the total volume in each tube was increased to 0.2 ml with assay buffer. Labeled CBG (1 ml) was then added to all assay tubes, which were mixed and incubated in a 40° C water bath for 5 minutes. The rack of tubes was immediately transferred to an ice-water bath for 15 minutes before the addition of 0.2 ml of cold (4° C) dextran-coated charcoal (0.5% charcoal Norit A, 0.5% dextran T-20, in assay buffer) to all assay tubes, which were mixed and incubated for a further



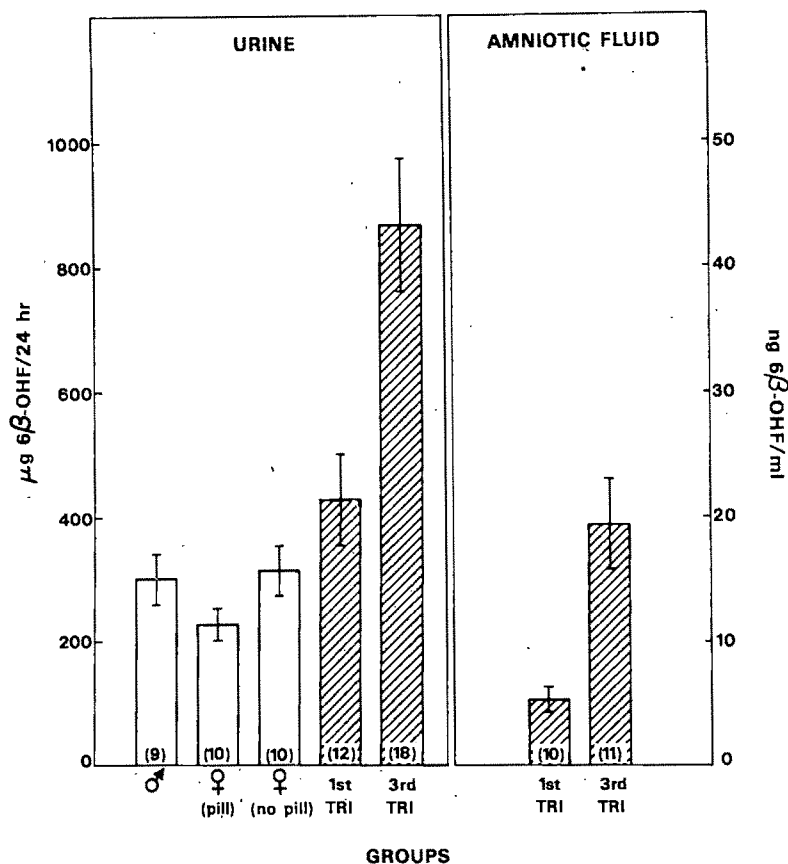


Fig. 3. Mean levels of 6 $\beta$ -OHF  $\pm$  1 SEM in urine and amniotic fluid of normal patients. The number of patients per group is indicated in parentheses. Groups: ♂, male subjects; (pill), female subjects receiving oral contraceptive medication; (no pill), female subjects not receiving estrogenic medication; 1st TRI, first trimester of pregnancy; 3rd TRI, third trimester of pregnancy.

10 minutes in the ice-water bath. All tubes were then centrifuged at 1,500  $\times$  g for 10 minutes at 4° C, and the decanted supernatant was counted for 5 minutes or to 1.5% error. An additional three vials containing 1 ml of labeled CBG and 0.4 ml of buffer were counted with each assay as a measure of the total counts added to the assay tubes. Values were read off the standard curve and then adjusted for the percentage recovery. Finally, urine results were converted to micrograms of 6 $\beta$ -OHF excreted per 24 hour and amniotic fluid results were expressed as nanograms of 6 $\beta$ -OHF per milliliter.

Assay specificity was validated by diluting some urine and amniotic fluid specimens with distilled water prior to extraction of 6 $\beta$ -OHF from a 5 ml aliquot of the specimens as described above. The standard 6 $\beta$ -OHF dose-response curves used for urine and amniotic fluid were then compared for parallelism with curves obtained from serial dilutions of urine and amniotic fluid containing known amounts of 6 $\beta$ -OHF.

**Statistical analyses.** Statistical differences in the

mean levels of 6 $\beta$ -OHF in urine and amniotic fluid of the various groups of patients were compared by one-way analysis of variance with the use of a Newman-Kuels range test for multiple means.<sup>19</sup>

## Results

**Standard curve.** Fig. 1 shows representative standard curves obtained in the radioassay of 6 $\beta$ -OHF in urine and amniotic fluid. The working range for the assay was 0 to 50 ng of 6 $\beta$ -OHF for urine and 0 to 20 ng of 6 $\beta$ -OHF for amniotic fluid.

**Sensitivity.** The sensitivity of the assay, defined as twice the standard deviation of the determination of zero on the standard curve, was 3.2 ng/ml for urine and 1.9 ng/ml for amniotic fluid.

**Precision.** An index of precision of the assay was established from replicate assays of pooled third-trimester urine samples. The intra-assay coefficient of variation for 12 urine samples assayed in triplicate, all on the same standard curve, was 12.5%. The interassay

coefficient of variation for 12 urine samples assayed in triplicate, each on a different standard curve on different working days, was 12.9%.

**Specificity.** The ethyl acetate extract of urine contained  $6\beta$ -OHF, other steroids, and an unidentified substance (X). The latter material could be detected when the extract was chromatographed on the column and all the eluted fractions were monitored by radioassay.

The elution profile of various steroids from a Sephadex LH-20 column, their identification by one or more methods, and their respective cross-reactivities with  $6\beta$ -OHF are listed in Table I. Only estriol (Fractions 18 to 21) eluted with  $6\beta$ -OHF (Fractions 16 to 20), but its cross-reactivity with  $6\beta$ -OHF was negligible. Fractions preceding  $6\beta$ -OHF contained several steroids which, if not separated chromatographically, would otherwise cross react to varying degrees with  $6\beta$ -OHF in the assay, the most notable being cortisol (Fractions 8 and 9) and substance X (Fractions 8 to 14).

Fig. 2 illustrates that the slopes of the dose-response curves for urine and amniotic fluid dilutions are highly similar to their respective assay slopes with the use of the purified  $6\beta$ -OHF standard.

**Urine and amniotic fluid analyses.** Fig. 3 shows the mean levels of  $6\beta$ -OHF  $\pm$  1 SEM in urine and amniotic fluid of various groups of normal patients as determined by the radioassay. Levels of  $6\beta$ -OHF in urine did not differ significantly between male subjects (300  $\mu$ g/24 hour) and nonpregnant female subject (313  $\mu$ g/24 hour) or those in the latter group who were receiving estrogenic medication (227  $\mu$ g/24 hour). During the first trimester of pregnancy, urinary levels of  $6\beta$ -OHF began to rise to 430  $\mu$ g/24 hour ( $p < 0.10$ ) and by the third trimester had increased markedly to 863  $\mu$ g/24 hour ( $p < 0.01$ ). Amniotic fluid levels of  $6\beta$ -OHF rose significantly from 5 ng/ml during the first trimester of pregnancy to 19 ng/ml during the third trimester ( $p < 0.002$ ).

### Comment

The assay described herein for the specific measurement of  $6\beta$ -OHF is of sufficient sensitivity to detect levels as low as 2 ng/ml and can be completed within 6 hours. Other binding proteins in rabbit, ovine, and human plasma did not prove to be as effective, when tested as an assay component, as did 0.5% or 1.0% dog plasma. Radiolabeling of the plasma CBG with  $^3\text{H}$ -cortisol rather than  $^3\text{H}$ - $6\beta$ -OHF increased the sensitivity of the assay, and we attributed this difference to the relatively low specific radioactivity of the  $^3\text{H}$ - $6\beta$ -OHF. The specificity of the present assay is achieved

first by extraction of extremely polar steroids by the use of ethyl acetate and second by separation of these extracted constituents from each other with a simple column-chromatography step. This is in contrast to previous attempts to quantitate  $6\beta$ -OHF, which required multidimensional paper chromatography and which ultimately depended on the general Porter-Silber chromagen reaction.<sup>3, 4, 17</sup> During the progress of our work a radioimmunoassay was reported for  $6\beta$ -OHF.<sup>20, 21</sup> The use of radioimmunoassay would afford greater sensitivity and the processing of larger numbers of specimens, although at least one method<sup>20</sup> nevertheless requires ethyl acetate extraction of specimens and separation of  $6\beta$ -OHF from cortisol by column chromatography. However, the assay system utilized in the present report has a limit of sensitivity well below the quantities of  $6\beta$ -OHF found in urine and amniotic fluid, and the 24-hour urine levels which we detected in male and nonpregnant female subjects are virtually identical with values obtained by radioimmunoassay.<sup>21</sup>

Present assay values for  $6\beta$ -OHF in urine specimens obtained from nonpregnant patients and during the third trimester of pregnancy are in agreement with values obtained by the earlier method.<sup>3</sup> Our finding that amniotic fluid levels of  $6\beta$ -OHF increase with advancing gestation suggests a potentially significant contribution by the fetus to the total  $6\beta$ -OHF pool. However, in contrast to earlier reports<sup>8, 9</sup> that estrogenic compounds can enhance urinary excretion of  $6\beta$ -OHF, we could not demonstrate this effect in patients who were receiving oral contraceptive medication.

There is evidence that, in addition to the very significant quantities of  $6\beta$ -OHF, trace amounts of other extremely polar steroids also exist in pregnancy urine, including a 20-hydroxysteroid derivative of  $6\beta$ -OHF.<sup>22</sup> The placenta is apparently a minor contributor to the production of  $6\beta$ -OHF or its 20-hydroxysteroid derivatives.<sup>23</sup> It may be that the steroid constituents of substance X are a composite of hydroxylated derivatives of cortisol and other steroids extracted by ethyl acetate. We conclude that substance X is not a glucuronide conjugate since its column elution profile was unaltered after treatment with  $\beta$ -glucuronidase.

Previous investigators<sup>4, 24, 25</sup> have noted that newborn infants excrete a high proportion of unconjugated cortisol metabolites in urine compared to the predominant glucuroconjugates excreted in children and adults. This difference was attributed to a relative immaturity of the hepatic glucuronosyl transferase system in neonates. In another study it was reported that urine levels of  $6\beta$ -OHF are markedly increased above normal



pregnancy levels in patients with toxemic pregnancy.<sup>3</sup> The present report on 6 $\beta$ -OHF offers the possibility of routine investigation of this major steroid metabolite in both urine and amniotic fluid of potential abnormal pregnancies and in selected endocrinopathies.

We thank Mrs. E. M. Peters for her technical assistance and Dr. S. Burstein, Mt. Sinai Hospital Medical School, New York, New York, for providing the reference standards of 2 $\alpha$ - and 2 $\beta$ -hydroxycortisol.

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# 6 $\beta$ -Hydroxycortisol levels in maternal urine of pregnancies complicated by prematurity

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Maternal urinary 6 $\beta$ -hydroxycortisol (6 $\beta$ -OHF) and creatinine levels were measured in 28 patients with singleton pregnancies admitted in apparent premature labor and in 17 women with singleton pregnancies delivered at term. Urine specimens were collected immediately after admission but prior to the initiation of active clinical intervention and management. Fourteen patients in true premature labor who were spontaneously delivered less than 24 hours after admission had significantly elevated 6 $\beta$ -OHF levels ( $1,144 \pm 10$  ng/ml) compared to levels in 14 patients in false premature labor who were delivered more than 48 hours after admission ( $544 \pm 88$  ng/ml). The mean ratio of 6 $\beta$ -OHF to creatinine was also elevated in patients in true premature labor (184.5) compared to ratios in patients delivered more than 48 hours after admission and in women in labor at or near term (range 123.4 to 128.6). The absolute levels of creatinine in all groups did not differ significantly, suggesting that the excretion of 6 $\beta$ -OHF proceeds independently of any change in renal function. Only in patients in the true premature labor group were 6 $\beta$ -OHF levels correlated with creatinine levels. However, in urine obtained within 24 hours prior to premature delivery, 6 $\beta$ -OHF levels did not appear to be related to the presence or absence of neonatal respiratory distress syndrome. It is concluded that measurement of 6 $\beta$ -OHF may provide an objective criterion for directing tocolytic therapy to those patients in true premature labor. (AM. J. OBSTET. GYNECOL. 139:250, 1981.)

RIISING FETAL CORTISOL levels in late pregnancy are responsible for a number of maturational events in preparation for labor.<sup>1</sup> Cortisol can induce enzymatic changes in the placenta, which lead to alterations in progesterone and 17 $\beta$ -estradiol levels immediately preceding labor.<sup>2</sup> Glucocorticoids are also responsible for differentiation of type II pneumocytes and their subsequent synthesis and release of surfactant.<sup>3</sup> Therefore, information about fetal cortisol production, obtained by a noninvasive technique, could have potential clinical applications in fetal assessment.

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Hepatic hydroxylation of cortisol serves to facilitate its metabolism and excretion as 6 $\beta$ -hydroxycortisol, which has been found to be a prevalent unconjugated polar metabolite in maternal urine, in the urine of neonates, and in amniotic fluid.<sup>4-6</sup> The maternal urinary excretion of 6 $\beta$ -hydroxycortisol (6 $\beta$ -OHF) during pregnancy has been attributed to the influence of estrogens,<sup>7, 8</sup> and it has been suggested that the high proportion of 6 $\beta$ -OHF in fetal and neonatal urine may be due to a relative immaturity of the glucuronosyl transferase system.<sup>5, 9, 10</sup> Furthermore, the immaturity of renal function in the fetus and early neonate could also tend to favor the excretion of unconjugated polar metabolites inasmuch as it has been shown that adult renal insufficiency impairs excretion of conjugated corticosteroids.<sup>11</sup> Thus, the fetus would appear to be a major contributor to the maternal pool of 6 $\beta$ -OHF and measurement of this polar metabolite could serve to differentiate, to a degree, cortisol excreted by the fetus from that originating in the mother.

The objectives of the present study were to determine the levels of 6 $\beta$ -OHF in maternal urine of pa-



**Table I.** 6 $\beta$ -OHF and creatinine levels in urine from patients in apparent premature labor and during or before term labor

|                              | Patients admitted in premature labor                 |                                                            | Patients delivered at term          |                                     |
|------------------------------|------------------------------------------------------|------------------------------------------------------------|-------------------------------------|-------------------------------------|
|                              | Patients delivered within 24 hours of admission (14) | Patients delivered more than 48 hours after admission (14) | Patients evaluated during labor (9) | Patients evaluated before labor (8) |
| 6 $\beta$ -OHF (ng/ml)       | 1,144 $\pm$ 210                                      | 544 $\pm$ 88                                               | 829 $\pm$ 341                       | 677 $\pm$ 237                       |
| Creatinine (mmoles/L)        | 6.20 $\pm$ 1.19                                      | 4.23 $\pm$ 0.64                                            | 6.72 $\pm$ 0.97                     | 5.33 $\pm$ 1.68                     |
| 6 $\beta$ -OHF<br>Creatinine | 184.5                                                | 128.6                                                      | 123.4                               | 127.0                               |

Values are means  $\pm$  SE. Number of patients per group is indicated in parentheses.

tients whose pregnancies were complicated by premature delivery and to determine the relationship between 6 $\beta$ -OHF levels and premature labor and the neonatal respiratory distress syndrome.

### Material and methods

**Patient selection.** All patients admitted to the Foothills Provincial General Hospital in Calgary over a 3-month period, in the spring of 1978, because of premature labor were asked to participate in this study. Each volunteer provided a voided urine specimen shortly following admission but prior to the initiation of active intervention to forestall the continuation of premature labor and delivery. For comparison, urine samples were obtained from women with normal term pregnancies—eight from women within 48 hours of normal delivery and nine from women in active labor.

For this report, only the results for the 28 women admitted in premature labor whose pregnancy resulted in the delivery of a normal living infant of low birth weight (less than 2,500 grams), whose labor was neither augmented nor terminated by caesarean section, were considered.

**Assay of urine specimens.** A specimen of urine voided immediately upon admission was obtained and a 5 ml aliquot was stored frozen at  $-20^{\circ}$  C. The assay for urinary 6 $\beta$ -OHF was performed according to the method of Bowler-Wong and associates.<sup>12</sup> In an attempt to relate 6 $\beta$ -OHF levels to renal function, urinary creatinine levels were determined on an S.M.A.C. autoanalyzer by means of a modification of the Jaffe reaction.

### Results

Of the 28 patients who were spontaneously delivered of normal infants of low birth weight, 14 were delivered within 24 hours of admission for premature labor and 14 were delivered 48 hours or more from the time of admission. For the latter group, the interval between admission and delivery varied from 2½ to 68 days, with

a mean of 31.4 days. Each group had received identical obstetric management on admission. The gestational age of the infant was considered compatible with the date of the last menstrual period and confirmed that the maternal urine sampling was done between 140 days (Alberta limit of viability) and 259 days (limit of prematurity). Within each group, 11 of the 14 neonates fell between the tenth and ninetieth percentiles of weight-for-dates criteria, as stated by Lubchenco and associates.<sup>13</sup>

Table I lists the levels of 6 $\beta$ -OHF and creatinine in urine from patients in apparent premature labor and during or before term labor. It can be seen that patients in premature labor who were delivered in less than 24 hours had significantly ( $p < 0.025$ ) elevated 6 $\beta$ -OHF levels (1,144  $\pm$  210 ng/ml) when compared to patients who were delivered more than 48 hours after admission (544  $\pm$  88 ng/ml). The 6 $\beta$ -OHF levels observed in patients before and during normal term labor were not significantly different.

Urine creatinine levels in patients with premature labor were not statistically different in the two groups delivered less than 24 or greater than 48 hours after admission. Similarly, urine creatinine levels did not change significantly before or during normal term labor.

In Table I the 6 $\beta$ -OHF/creatinine ratios of the various groups are compared. It can be seen that the ratio remains relatively constant (123.4 to 128.6) in three groups but is greatly elevated (184.5) in those patients with true premature labor delivered less than 24 hours after admission. In this latter group we also observed a significant correlation ( $r = 0.81$ ,  $p < 0.001$ ) between levels of 6 $\beta$ -OHF and creatinine. No such correlation was observed in the other three groups.

Birth weights and gestational ages of all premature infants were each independently examined relative to 6 $\beta$ -OHF excretion and no correlations were found. There did not appear to be any trends between the presence or absence of neonatal respiratory distress in

the infants evaluated by chest x-ray film and the  $6\beta$ -OHF levels in maternal urine obtained within 24 hours prior to delivery.

### Comment

The findings in the present investigation clearly indicate that elevated maternal levels of  $6\beta$ -OHF can portend delivery in those patients who are admitted with true premature labor. Because collection of 24-hour urine specimens was not possible in these patients, the urine creatinine determinations were used as an index of renal function. Although there was a tendency for urine creatinine levels to change during labor, the shift was not significant and the ratio of  $6\beta$ -OHF to creatinine remained constant in all groups except the group in true premature labor. This would suggest that excretion of  $6\beta$ -OHF proceeds independent of any possible change in renal function. It is interesting to note that  $6\beta$ -OHF and creatinine levels in urine were correlated only in the group of 14 patients in true premature labor. As speculation we suggest that this finding may represent a maximum clearance of  $6\beta$ -OHF associated with true premature delivery. Furthermore, we cannot account for these differences in  $6\beta$ -OHF excretion on the basis of variation in gestational ages of the two populations admitted as candidates for possible premature delivery, because normal pregnancies sampled serially throughout gestation show elevated but relatively constant levels of  $6\beta$ -OHF excretion between 217 and 241 days of gestation (unpublished data).

Although the fetal versus maternal contributions to the  $6\beta$ -OHF pool are difficult to resolve, the very significant increase in amniotic fluid  $6\beta$ -OHF levels which we have observed as gestation advances<sup>12</sup> is consistent with the explanation offered by others,<sup>5, 9, 10</sup> that the

fetus and neonate excrete significant quantities of  $6\beta$ -OHF in lieu of glucuronide-conjugated cortisol because of an immature glucuronosyl transferase system. Therefore, it would seem possible that short-term changes in urinary levels of  $6\beta$ -OHF could reflect alterations in the fetal excretory pattern of this polar metabolite secondary to changes in fetal adrenal cortisol formation. We have noted normal decreasing levels of  $6\beta$ -OHF in amniotic fluid in late pregnancy (unpublished data) which may represent an increasing maturation of the glucuronosyl transferase system as term approaches. This could explain the differences in  $6\beta$ -OHF excretion between patients in premature labor versus those in term labor observed in the present investigation.

It is well established that following a rise in exogenous corticosteroid levels there is a delay in the activation of the enzyme system involved in the production of surfactant.<sup>14, 15</sup> Other factors in addition to glucocorticoid levels are also responsible for manifestation of the respiratory distress syndrome.<sup>16</sup> Therefore, our inability to predict the occurrence of respiratory distress syndrome on the basis of  $6\beta$ -OHF levels may be due to a variety of causes, including insufficient time for allowing metabolic processes to develop in infants who were delivered less than 24 hours after admission.

In conclusion, further investigation would seem warranted in view of the evidence that elevated  $6\beta$ -OHF levels may identify those fetuses particularly likely to be delivered within 24 hours, thereby permitting re-evaluation of tocolytic therapy and its direction toward appropriate patients in true premature labor.

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# Qualitative amniotic fluid volume determination by ultrasound: Antepartum detection of intrauterine growth retardation

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Qualitative amniotic fluid volume (AFV) was determined by a linear array ultrasound method in 120 patients referred with a diagnosis of intrauterine growth retardation (IUGR). Eighty-nine of these patients were delivered of a normal infant; the false positive rate by clinical methods alone was 74.2%. Qualitative AFV was termed normal if at least one pocket of amniotic fluid measuring 1 cm in its broadest diameter was identified. Qualitative AFV was normal in 91 patients, and 86 were delivered of a normal fetus (93.4%). In contrast, qualitative AFV was decreased in 29 patients, and 26 were delivered of a fetus with IUGR (89.9%). These differences were highly significant. Perinatal morbidity was increased tenfold in patients with decreased AFV. Overall, 26 of 31 IUGR fetuses (83.4%) demonstrated decreased qualitative AFV. (AM. J. OBSTET. GYNECOL. 139:254, 1981.)

INTRAUTERINE growth retardation (IUGR), defined as a birth weight of less than the tenth percentile for gestational age and sex, complicates up to 10% of all pregnancies and is associated with a sixfold to eightfold increase in perinatal mortality and morbidity.<sup>1</sup> In a recent study, Morrison and associates<sup>2</sup> reported that IUGR was present in 7.5% of perinatal deaths occurring in the Province of Manitoba. Despite recognition of the high prevalence and serious nature of this complication, less than 40% of infants with IUGR are recognized before delivery.<sup>3</sup> IUGR has been broadly classified into two categories: IUGR resulting from an early embryonic insult with decreased cell number; and IUGR resulting from impaired cell growth secondary to reduced metabolic and nutritive placental func-

tion.<sup>4-6</sup> Whereas the former category (decreased cell number) is rarely amenable to therapy, the latter category (uteroplacental insufficiency) may be effectively treated by appropriate delivery of the infant.<sup>7</sup>

Serial ultrasound measurement either of fetal growth parameters (e.g., biparietal diameter) or of uterine growth (total intrauterine volume) has been proposed as the means for detecting the fetus with IUGR.<sup>3, 8</sup> Other authors<sup>9</sup> have reported measurement of the head/abdomen ratio as a useful method for detecting IUGR. Each of these methods is based on a nomogram relating the given variable (e.g., biparietal diameter growth) to gestational age. Unfortunately, since absolute gestational age may be in doubt in up to 40% of normal pregnancies, and in an even higher proportion of those in which IUGR is suspected, practical clinical application of those ultrasound morphometric methods may be difficult. Thus, Campbell and associates,<sup>3</sup> for example, reported only a 12% improvement in detection of IUGR on the basis of serial biparietal diameters as compared to clinical means alone.

Oligohydramnios has long been recognized as a clinical hallmark of dysmature IUGR. Amniotic fluid is readily identified by ultrasound. In a recent study, qualitative amniotic fluid volume (AFV) as determined

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by a real-time ultrasound method proved to be useful in screening for IUGR.<sup>10</sup> In this high-risk pregnant population, 20 of 21 infants with IUGR (96%) demonstrated decreased qualitative AFV (<1 cm pockets).<sup>10</sup> These observations prompted this prospective study designed to determine the value, if any, of qualitative AFV in patients in whom there was clinical suspicion of IUGR.

## Method

### Qualitative amniotic fluid volume determinations.

Study patients were scanned in a supine position by means of a linear array real-time ultrasound method\* with a 3.5 megahertz transducer. The entire uterus was scanned to determine fetal lie and attitude. Pockets of amniotic fluid were identified and the size of the pockets was determined by freeze-frame mode and electronic calipers. Pockets were measured in their broadest diameter. In most instances, the largest pockets of amniotic fluid were found in the region of the fetal limbs. Classification of qualitative AFV as normal or abnormal was based on a previously reported blind study that demonstrated a significant increase in the incidence of IUGR in fetuses with pockets of amniotic fluid of less than 1 cm.<sup>10</sup> Accordingly, in this study, qAFV was coded normal if any pockets exceeded 1 cm in the broadest diameter. Patients with the largest pockets of amniotic fluid measuring less than 1 cm in the broadest diameter were coded as having decreased fluid. No attempt was made to measure serial change in AFV since the size of this pocket varied with fetal position.

**Study population.** The study population consisted of 150 consecutive patients referred with a clinical diagnosis of IUGR based upon a discrepancy of at least 4 weeks' size between gestational age and fundal height. At the time of referral, a repeat history was obtained and physical examination was performed. Patients with obviously mistaken dates or decreased fundal height because of transverse lie were excluded from the study. The remainder of the patients were classified as having suspected IUGR.

**Management protocol.** In this study, management of patients with suspected IUGR was based entirely on qualitative AFV determination (Fig. 1). Patients with normal qAFV were classified as having mistaken dates, and the gestational age was adjusted according to the probable last normal menstrual period (LNMP). Repeat examination was not performed in these patients.

\*Advanced Diagnostic Research, Ltd., Model 2130 AA, Phoenix, Arizona.

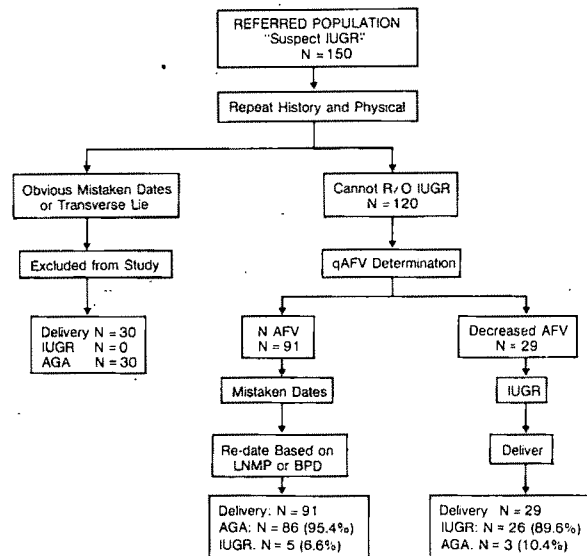


Fig. 1. Management protocol in suspected IUGR.

Table I. Perinatal mortality and morbidity

| Variable | N  | 5 Minute Apgar <7* |     | Fetal distress in labor* |     | Perinatal mortality |                |
|----------|----|--------------------|-----|--------------------------|-----|---------------------|----------------|
|          |    | N                  | %   | N                        | %   | N                   | Rate per 1,000 |
| AGA      | 89 | 2                  | 2.2 | 3                        | 3.3 | 0                   | 0              |
| IUGR     | 31 | 6                  | 22† | 9                        | 33† | 4                   | 33.3/1,000     |

AGA: Appropriate for gestational age.

\*Per live births.

†p < 0.05 chi square.

Patients with decreased qualitative AFV were considered to probably have IUGR, and immediate delivery was recommended. Exceptions to this protocol were patients with recognized fetal congenital anomaly or gross immaturity (<28 weeks).

All infants were weighed and examined at delivery, and were considered to be growth retarded if the birth weight was less than the tenth percentile for gestational age and sex, with use of a sea-level nomogram.<sup>11</sup>

The gestational age for birth weight percentile was determined either by LNMP, when reliable, or by Dubowitz score,<sup>12</sup> when dates were unsure. In case of doubt, the Dubowitz score was taken as the final determinant of gestational age. In infants whose weight was less than the tenth percentile, signs of dysmature IUGR, such as decreased subcutaneous fat, hypoglycemia, and polycythemia, were noted. The incidence of fetal distress in labor (defined as emergency termination of labor), 5 minute Apgar score, and perinatal mortality were recorded.

**Table II.** Perinatal mortality

| Name  | Gestational age (wk) | Birth weight (gm) | Percentile | Comment                            |
|-------|----------------------|-------------------|------------|------------------------------------|
| C. D. | 34                   | 1,430             | <3         | Renal agenesis, fetal death        |
| C. T. | 32                   | 1,030             | <5         | Renal agenesis, fetal death        |
| I. F. | 34                   | 1,250             | <3         | Renal agenesis, intrapartum death  |
| E. P. | 27                   | 600               | <3         | Fetal death, grossly normal infant |

**Table III.** Incidence of IUGR in patients with "suspected" IUGR

| Method                 | No. of patients | % IUGR |       | False positive rate (%) | False negative rate (%) |
|------------------------|-----------------|--------|-------|-------------------------|-------------------------|
|                        |                 | N      | %     |                         |                         |
| Clinical estimate only | 120             | 31     | 25.8  | 74.2                    | —                       |
| Qualitative AFV        |                 |        |       |                         |                         |
| Normal (>1 cm)         | 91              | 5      | 6.6   | —                       | 6.6                     |
| Decreased (<1 cm)      | 29              | 26     | 89.6* | 10.4                    | —                       |

\*p &lt; 0.001 by chi-square test.

### Results

One hundred fifty consecutive patients were referred with a clinical diagnosis of IUGR. Thirty of these patients (20%) were excluded from this study because of obvious mistaken dates (N = 26) or transverse lie (N = 4). None of the 30 patients who were excluded was delivered of an infant with IUGR. The other 120 patients were considered to be at risk for IUGR and form the basis of this report. The gestational age of these patients at the time of referral ranged from 27 to 40 weeks. All of these patients had a minimum of 4 weeks' discrepancy between clinical size and dates (mean,  $5.6 \pm 1$  week; range, 4 to 12 weeks). In 81 patients (67%) the LNMP was known, and in the other 39 patients the LNMP was unknown (33%). Twenty per cent of patients were first examined before 12 weeks' gestation, 36% between 12 and 26 weeks' gestation, and 44% after 26 weeks' gestation. One hundred five patients were multiparous (87.1%), and seven of these (6.6%) had previously been delivered of an infant with IUGR. Most of the patients studied (80%) had historical high-risk factors (e.g., hypertension).

Thirty-one patients were delivered of an infant with IUGR (25.8%). The false positive rate by clinical means alone was 74.2% (89 of 120 patients). Twenty-six of 31 infants with IUGR weighed less than the fifth percentile for gestational age and sex (86.5%), and the remainder weighed between the fifth and tenth percentiles. The mean gestational age for infants with IUGR was  $37.2 \pm 1.2$  SEM weeks, as compared to  $38.8 \pm 1.5$  SEM weeks for normal infants (not sig-

nificant by Student's t test). The incidences of fetal distress in labor, low 5 minute Apgar score, and perinatal mortality were significantly increased in infants with IUGR (Table I). There were four perinatal deaths in the study group (33.3 per 1,000), and all occurred in infants with IUGR (Table II). All but one of these deaths were directly attributed to congenital anomalies. The corrected perinatal mortality was 8.3 per 1,000 live births.

### Qualitative amniotic fluid volume (Table III).

**Normal qualitative AFV (>1 cm pocket).** Qualitative AFV was recorded as normal in 91 of 120 study patients (75.8%); 86 subsequently were delivered of an infant appropriate for gestational age (93.4%). The mean interval between last observation and delivery was  $19.1 \pm 3$  SEM days (range, 0 to 60 days). Five of 91 patients with normal qualitative AFV were delivered of an infant with IUGR (false negative rate 6.6%). All five infants with IUGR were delivered within 7 days of the last observation. Three of these five infants with IUGR weighed less than the fifth percentile (60%). Four of these five infants were delivered vaginally, uneventfully; and the fifth infant was delivered by emergency lower-segment cesarean section because of prolonged bradycardia and was found to have a true knot in the cord. None of these five infants developed neonatal morbidity. The mean duration of hospital stay was  $4.0 \pm 1$  day (range, 3 to 7 days).

**Decreased qualitative AFV (<1 cm pocket).** Qualitative AFV was recorded as decreased in 29 of 120 patients (24.2%); 26 of these patients were delivered of an infant with IUGR (89.6%). The incidence of IUGR was significantly increased in these patients as compared to patients with normal qualitative AFV (p < 0.001 chi square). The mean interval between last observation and delivery was  $3.9 \pm 5$  days (range, 0 to 35 days). Delivery was delayed in four patients, in one instance because of gross immaturity (27 weeks) and in three instances because of recognized congenital anomalies (Table I). All of these infants died in utero. Labor was induced in the other 25 patients with decreased qualitative AFV; in 11, development of fetal distress required delivery by cesarean section (44%) and all infants were growth retarded. Nineteen of the 25 surviv-



ing fetuses (76%) developed neonatal morbidity (e.g., hypoglycemia). Mean duration of hospital stay for surviving infants was  $7.7 \pm 2.0$  days (range, 3 to 16 days).

### Comment

Despite an increasing awareness of the importance of IUGR as a cause of perinatal mortality and morbidity, antepartum detection of this complication remains poor. Identification of the high-risk fetus by clinical methods alone is difficult because of inaccuracies in estimates of true gestational age and weight of the fetus. In this study, 91 of 120 women with clinical suspicion of IUGR (74.8%) were delivered of normal infants. In contrast, Campbell<sup>3</sup> reported detection of only 35 of 115 (28.7%) growth-retarded infants by clinical methods alone. Thus, the sole use of clinical methods to detect IUGR appears to be inadequate.

Oligohydramnios is a clinical hallmark of dysmature IUGR. In this study, 26 of 31 growth-retarded fetuses (83.9%) had ultrasound evidence of decreased amniotic fluid. The etiology of this phenomenon is speculative. Experimental chronic uteroplacental insufficiency in fetal lambs and monkeys is associated with an increased incidence of IUGR.<sup>13, 14</sup> Fetal hypoxemia is characteristically observed in these fetuses. Similarly, cord blood  $PO_2$  is reported to be significantly reduced in growth-retarded human fetuses.<sup>15</sup> In the second half of pregnancy, fetal urine and lung liquid are major contributors to the amniotic fluid pool.<sup>16</sup> Experimental hypoxemia in chronically instrumented fetal lambs results in reflex redistribution of cardiac output, so that renal blood flow falls dramatically and pulmonary blood flow nearly ceases.<sup>17</sup> We postulate that one mechanism for oligohydramnios in growth-retarded human fetuses may be a decreased production of fetal urine and lung liquid as a result of hypoxemia-induced redistribution of cardiac output. This hypothesis is supported by reports of a decreased production of fetal urine and decreased fetal breathing movements in human fetuses with IUGR.<sup>18, 19</sup> In accordance with this hypothesis, qualitative AFV determination may be viewed as a functional measure of the effect of IUGR. This concept is important since it implies that screening for IUGR by this method may be largely independent of the gestational age of the fetus. In contrast, ultrasound morphometric measurements of IUGR, such as determination of total intrauterine volume and head/abdomen circumference ratio, require precise knowledge of gestational age for interpretation. Combined functional and morphometric measurements in patients with known gestational age are likely to be complementary.

In this study, qualitative AFV determination by a real-time ultrasound method was useful in differentiating the normal from the growth-retarded fetus. Patients with normal qualitative AFV were delivered of a normal infant in 93.4% of instances, whereas those with decreased qualitative AFV were delivered of a fetus with IUGR in 89.4% of instances. These differences were highly significant. These findings also indicate that this method may identify the fetus at risk for perinatal morbidity and mortality, since patients with decreased qualitative AFV experienced a tenfold increase in fetal distress and neonatal depression, and all perinatal deaths occurred in this group. In addition, the duration of hospitalization for neonates with decreased qualitative AFV was prolonged ( $7.7 \pm 2$  days), as compared to that for infants with normal qualitative AFV ( $2.0 \pm 1$  day). The high sensitivity (false negative rate of 6.6%) and specificity (false positive rate of 10.4%) of the method and the rapidity and ease in its application make screening of high-risk populations a practical venture.

In this study, we observed five patients with normal qualitative AFV who were delivered of fetuses with IUGR. We cannot explain these false negative results. Changes in amniotic fluid volume with advancing gestational age may represent one source of error. However, the high true positive rate of IUGR (89.6%) in patients with decreased AFV over a wide range of gestational age (range, 27 to 42 weeks) indicates that these changes are unlikely to be a major source of error. It is noteworthy that none of these five false negative results was associated with neonatal morbidity, whereas 19 of 25 surviving growth-retarded fetuses with decreased qualitative AFV (76%) developed neonatal morbidity. Since percentile ranking by birth weight does not indicate a pathologic state per se, it is possible that a proportion of these false negative results represented normal "small" infants. We have also observed three infants who, despite decreased qualitative AFV, were normal at birth. Again, we cannot explain these false positive findings. Further studies on the mechanisms of oligohydramnios and rate of development in IUGR fetuses may provide an explanation for these false positive and false negative results. In addition, we have observed a high incidence of renal agenesis in patients suspected of IUGR and oligohydramnios (13.8%). The high rate of this lethal congenital anomaly emphasizes the need for complete ultrasound examination of patients with oligohydramnios.

A reasonable assumption seems to be that the accurate detection of IUGR and early intervention may reduce the high perinatal mortality and morbidity rate

associated with this disorder. These data indicate that qualitative AFV screening may be of value in the identification of these fetuses, and, in particular, the fetuses in whom gestational age is unknown. Combination of

this functional method for detecting IUGR with conventional morphometric measurements is likely to further enhance the recognition of these high-risk fetuses.

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# Phosphatidylglycerol determination on amniotic fluid 10,000 × g pellet in the prediction of fetal lung maturity

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Phosphatidylglycerol and the lecithin/sphingomyelin (L/S) ratio were determined, and the shake test was performed, as indicators of fetal lung maturity, in more than 600 patients. A clinical review of the outcome was made in all patients who underwent delivery within 2 days after collection of amniotic fluid. Correlation was made between phosphatidylglycerol, shake test, and L/S ratio results. L/S ratio had a false positive rate of 5%, and a false negative rate of 58.1%. The shake test had a false positive rate of 1.1%, and a false negative rate of 63.5%. Phosphatidylglycerol determination had a false positive rate of 1.8%, and a false negative rate of 26.9%. Phosphatidylglycerol determination is an accurate predictor of fetal lung maturity, and predicts lung immaturity more correctly than do the L/S ratio and shake test. (AM. J. OBSTET. GYNECOL. 139:259, 1981.)

A CONTINUING NEED exists in the management of high-risk pregnancy for a precise test of fetal pulmonary maturity, which accurately predicts lung immaturity as well. Lung surfactant is present in amniotic fluid as intact lamellar bodies which can be quantitatively harvested as a pellet fraction by centrifugation at 10,000 × g.<sup>1, 2</sup> Since January, 1978, at this institution, in addition to the routine analysis of the lecithin/sphingomyelin (L/S) ratio and the shake test as indicators of fetal lung maturity, phosphatidylglycerol (PG) has been measured in the isolated pellet fraction.

The L/S ratio and shake test are reliable predictors of fetal lung maturity,<sup>3-7</sup> except in diabetic mothers, whose infants have an increased incidence of respira-

Table I. Data base

|                                    | Total | Delivery within 2 days of fluid collection |
|------------------------------------|-------|--------------------------------------------|
| No. of PG determinations           | 636   | 244                                        |
| No. of shake test on same specimen | 628   | 235                                        |
| No. of L/S ratios on same specimen | 408   | 103                                        |

tory distress syndrome (RDS) despite a mature test result.<sup>3, 5, 6, 8-10</sup> Both tests, however, lack predictive accuracy in that a negative result correlates poorly with fetal lung immaturity. Such a false negative result may lead to unnecessary prolongation of a high-risk pregnancy, or unnecessary administration of steroids.

The major etiologic factor in the development of RDS is a deficiency of pulmonary surfactant<sup>11-13</sup> which contains more than 50% dipalmitoyl lecithin.<sup>14, 15</sup> Mature surfactant, i.e., surfactant which contains PG, has better alveolar stabilizing properties than does surfactant which has not yet acquired PG.<sup>11, 16</sup> In the absence of PG, the risk of RDS is significant, although development of the disease is not inevitable.<sup>11, 17, 18</sup> An added advantage of measuring PG in amniotic fluid is its virtual absence from blood, meconium, and vaginal se-

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**Table II.** Results of PG, L/S ratio, and shake test

| Test       | Result   | Total No. of cases | RDS (No.) | False negative (%) | False positive (%) |
|------------|----------|--------------------|-----------|--------------------|--------------------|
| L/S        | <2       | 43                 | 18        | 58.1               | —                  |
|            | ≥2       | 60                 | 3         | —                  | 5                  |
| Shake test | Negative | 52                 | 19        | 63.5               | —                  |
|            | Positive | 183                | 2         | —                  | 1.1                |
| PG         | Absent   | 26                 | 19        | 26.9               | —                  |
|            | Present  | 218                | 4         | —                  | 1.8                |

cretions, all of which contain phospholipids and other components which interfere with the measurement of the L/S ratio and shake test.<sup>2, 4, 19</sup> In specimens contaminated with these substances the most accurate measure can be made by isolating the 10,000 × g pellet fraction, which eliminates the contaminating phospholipids from the analysis.<sup>2</sup>

Several centers have measured phospholipids, including phosphatidylglycerol, in amniotic fluid,<sup>1-9, 20-23</sup> but the varying methodologies have made both the interpretation and comparison of the data confusing. In those studies, the presence of PG in amniotic fluid usually indicated fetal pulmonary maturity, although PG was often demonstrable only when the L/S ratio and shake test were mature. The analyses herein reported were performed on the lamellar body fraction, isolated by centrifugation of the amniotic fluid sample at 10,000 × g, and therefore represent a specific measure of fetal pulmonary surfactant.<sup>24</sup>

#### Material and methods

Samples of amniotic fluid were collected for the usual clinical indications.

The shake test was performed by the method of Clements and associates.<sup>25</sup>

The L/S ratio measurement was a modification of the method of Gluck and Kulovich,<sup>26</sup> with a value of 2 or greater indicating pulmonary maturity.

The procedure for the determination of PG was commenced by centrifugation of amniotic fluid at 140 × g for 5 minutes to remove cells and cellular debris, and centrifugation of the resultant supernatant at 10,000 × g for 20 minutes to sediment surfactant particles. The resulting supernatant was discarded and lipids were extracted from the pellet by suspension in 3 ml of 0.85% NaCl and extraction with one volume of methanol and two volumes of chloroform.<sup>2</sup> The lipid extract was dried, dissolved in 20 μl of chloroform: methanol (2:1 volume), and applied to a silica gel G-plate for development of two-dimensional, thin-layer chromatography with the use of chloroform: methanol: water (65:25:4 by volume) in the first di-

mension and butanol: acetic acid: water (90:30:30 by volume) in the second dimension.<sup>24</sup>

After air-drying the plates, phospholipids were visualized with a molybdenum-blue spray reagent.<sup>27</sup> Each phospholipid was scraped from the plate, its phosphorus content was measured,<sup>28</sup> and the percentage of PG was calculated. The presence of PG was interpreted as being a positive test.

Correlation of test result with neonatal outcome was based on independent neonatal assessment by staff neonatologists. Criteria for the diagnosis of RDS included the clinical findings of grunting, tachypnea, sternal and intercostal retractions, and nasal flaring. Symptoms must have persisted longer than 6 hours and required oxygen therapy.

Gestational age was calculated from the onset of the last normal menstrual period and checked by neonatal assessment. If neonatal assessment was within 2 weeks of determination by dates, the latter were considered to be accurate. Otherwise, the neonatal assessment of gestation was accepted.

#### Results

A total of 636 PG determinations was made (Table I). Of these, 244 were followed by delivery within 2 days of collection of amniotic fluid. Comparisons between PG, L/S ratio, and shake test include only this latter group of 244 cases.

A false positive test was a result which predicted lung maturity, but the infant developed RDS. A false negative result predicted lung immaturity, but the infant did not develop RDS (Table II).

The false positive rate for L/S ratio was 5%, and two of three cases occurred in diabetic pregnancies. The false negative rate was 58.1%.

The false positive rate for the shake test was 1.1%. One case was a diabetic pregnancy. The second case occurred in a patient who required cesarean section for premature labor at 35 weeks' gestation. The false negative rate for the shake test was 63.5%.

The false positive rate for PG determination was 1.8%. Three of the four cases occurred in diabetic



Table III. PG, L/S ratio, and outcome

|            | L/S ratio |       |         |    |     | Total |
|------------|-----------|-------|---------|----|-----|-------|
|            | <1        | 1-1.4 | 1.5-1.9 | ≥2 | ND* |       |
| PG present | 0         | 8     | 14      | 58 | 138 | 218   |
| RDS†       | —         | 0     | 2†      | 2‡ | 0   | 4     |
| PG absent  | 9         | 7     | 5       | 2  | 3   | 26    |
| RDS        | 8         | 4     | 5       | 1  | 1   | 19    |

\*Not determined.

†Diabetes mellitus in one patient.

‡Diabetes mellitus in both patients.

Table IV. PG, shake test, and outcome

|            | Shake test |              |          |     | Total |
|------------|------------|--------------|----------|-----|-------|
|            | Negative   | Intermediate | Positive | ND* |       |
| PG present | 13         | 17           | 180      | 8   | 218   |
| RDS        | 1†         | 1†           | 1†       | 1   | 4     |
| PG absent  | 18         | 4            | 3        | 1   | 26    |
| RDS        | 14         | 3            | 1        | 1   | 19    |

\*Not determined.

†Diabetes mellitus.

pregnancies in which the infants developed mild or moderate RDS, and made a good recovery. The only false positive result in a nondiabetic pregnancy occurred in a multiparous patient with premature rupture of membranes of more than 7 days. The week prior to delivery, she had received steroids to promote fetal lung development. The infant was delivered at 33 weeks and 2 days of gestation, and was not asphyxiated at birth. The infant had positive blood cultures in the septic work-up and developed mild RDS.

The false negative rate for PG determination was 26.9%.

PG was present in 22 cases in which the L/S ratio was immature, and two infants developed RDS (Table III). One infant was born to a diabetic mother, and the other was described above as the only case of a non-diabetic PG false positive. PG was absent in two cases when the L/S ratio was mature, and one infant developed severe RDS. RDS developed in 17 of 21 cases with absent PG and an L/S ratio of less than 2.

PG was present in 30 cases with a negative shake test (Table IV), and two infants developed RDS. Both of the mothers were diabetics. PG was absent in three cases when the shake test was positive, and one of these infants developed mild RDS. PG was absent in 22 cases with negative shake test, and 17 infants developed RDS.

PG was measured in 32 cases when both the shake test and L/S ratio indicated lung immaturity (Table V).

Table V. PG and outcome in cases with L/S ratio <2, shake test negative

|            | No RDS | RDS | Total |
|------------|--------|-----|-------|
| PG present | 11     | 1*  | 12    |
| PG absent  | 4      | 16  | 20    |

\*Diabetes mellitus.

Table VI. PG, L/S ratio, and shake test in gestational diabetics (Class A)

| Test       | Result   | Total No. of cases | RDS |
|------------|----------|--------------------|-----|
| PG         | Absent   | 1                  | 1   |
|            | Present  | 26                 | 0   |
| L/S        | <2       | 7                  | 1   |
|            | ≥2       | 9                  | 0   |
| Shake test | Negative | 5                  | 1   |
|            | Positive | 22                 | 0   |

Table VII. PG, L/S ratio, and shake test in patients with overt diabetes (White Classes B to R)

| Test       | Result   | Total No. of cases | RDS |
|------------|----------|--------------------|-----|
| PG         | Absent   | 1                  | 1   |
|            | Present  | 16                 | 3   |
|            | > 0 <1%  | 8                  | 3   |
|            | ≥1%      | 8                  | 0   |
| L/S        | <2       | 3                  | 2   |
|            | ≥2       | 14                 | 2   |
| Shake test | Negative | 5                  | 3   |
|            | Positive | 12                 | 1   |

Of 12 cases with PG present, only one infant developed RDS, and this was the infant of a diabetic mother. PG was absent in the other 20 cases, and 16 infants developed RDS.

In an additional 10 fluids when an L/S ratio of less than 2 was attributed to the presence of blood in the sample, PG was present and no infant developed RDS.

Infants of mothers with gestational diabetes did not develop RDS if any of the three tests indicated pulmonary maturity (Table VI). In overt diabetics (Table VII), a small number of infants developed RDS in the presence of PG. However, if a PG value of 1% were accepted as a positive test result in diabetic pregnancies, there were no false positives in our small number of cases in which delivery occurred within 2 days of collection of amniotic fluid.

Comment

The presence of PG in the isolated lamellar body fraction (10,000 × g pellet) of amniotic fluid is a reliable indicator of fetal pulmonary maturity. A mature L/S ratio and shake test were virtually always associated

with the presence of PG. However, PG was also present in many cases in which there was an immature shake test or L/S ratio, and unnecessary delays in delivery were reduced.

Surfactant is localized in the lamellar body fraction of amniotic fluid, and if centrifugation beyond  $140 \times g$  is used in the initial preparatory step, large quantities of these structures will be removed. When L/S ratios are determined on the supernatant, the frequency of false negatives may be attributed to this effect.<sup>2</sup> Centrifugation also results in the loss of PG, so that the small quantities of this phospholipid which are present before 37 weeks' gestation may not be detected in the supernatant fluid. This problem is avoided by isolating the lamellar body fraction as a  $10,000 \times g$  pellet and determining its surfactant composition directly.

In nondiabetics and gestational diabetics, the presence of PG even in trace amounts insures that the infants will not develop RDS. Some infants of overt diabetics developed RDS when PG was present at levels of less than 1% of the total phospholipids, but none did so when PG exceeded this value. We also noticed that the L/S ratio in diabetics was usually greater than 2 before PG appeared. The significance of this is not clear but might be clarified with a better understanding of the exact role of PG.

The absence of PG resulted in a greater than 70%

risk of the development of RDS. When PG was absent and the infants did not develop RDS, phospholipid analysis of the  $10,000 \times g$  pellet fraction indicated that maturation had already progressed to the last stage of surfactant production (early lamellar body formation) prior to the appearance of PG.<sup>24</sup>

On the basis of the findings in this study, this obstetric service, where there are 5,000 deliveries annually, has discontinued the use of L/S ratios. PG determination accurately predicts pulmonary maturity, gives a better indication of pulmonary immaturity than does the L/S ratio ( $p < 0.05$ ), and can be performed in less time. The test of pulmonary maturity is carried out as follows: (1) A shake test is performed on clear amniotic fluid, and if negative, the presence or absence of PG is determined on the  $10,000 \times g$  pellet. (2) When amniotic fluid is contaminated with blood, meconium, or vaginal mucus, PG is determined. (3) All overt diabetic patients require measurement of PG as a percentage of total phospholipids, with a value of 1% or greater indicating pulmonary maturity.

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# Hyperprolactinemia—A significant factor in female infertility

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A prospective study was undertaken to determine the incidence of hyperprolactinemia in a group of referred infertile women, and to determine the association with (a) abnormal menstrual function and (b) presence or absence of galactorrhea. Of 13 referred infertile patients, 22 (19.5%) had elevated levels of serum prolactin. Five (4.4%) patients with hyperprolactinemia had neither abnormal menstrual function nor galactorrhea. This study demonstrates that hyperprolactinemia is a common finding in an infertile population, more so when galactorrhea and/or menstrual dysfunction is also present. (AM. J. OBSTET. GYNECOL. 139:264-1981.)

HYPERPROLACTINEMIA in association with the galactorrhea/amenorrhea syndrome is a recognized cause of anovulatory infertility.<sup>1</sup> Several recent reports have related infertility to hyperprolactinemia with lesser degrees of menstrual dysfunction,<sup>2-5</sup> citing mainly corpus luteum deficiency as the cause of the infertility.<sup>2-5</sup> There is abundant animal and human physiologic research to establish a theoretical explanation for such a situation.<sup>1, 6-9</sup>

This prospective study was undertaken in order to establish whether the measurement of serum prolactin would be of benefit in all infertile women.

## Material and methods

One hundred and thirteen female patients referred to the Infertility Clinic for primary or secondary infertility over a period of 6 months formed the study group. All had been infertile for more than 1 year with adequate coital exposure. Most had undergone some initial investigation prior to referral.

At the initial visit, a complete history was taken and a physical examination was performed, including menstrual, obstetric, contraceptive, medical, surgical, and

drug ingestion data. Special attention was given to elucidating galactorrhea. Blood was drawn from all patients at the initial visit for determination of thyroxine ( $T_4$ ),  $T_3$ -resin uptake ( $T_3$ RU), free thyroxine index (FTI), thyroid stimulating hormone (TSH), and prolactin (Prl). Prl was measured by a double antibody radioimmunoassay (RIA). Reagents were obtained from Dr. H. Freisen, Winnipeg.

**Controls.** Normal levels of serum prolactin were based on 30 women between 18 and 25 years of age who had normal cycles (26 to 30 days). Half were parous, and none was ingesting drugs. Blood was drawn on two separate occasions in the early to mid-follicular phase of the cycle. The mean value was 12.5 ng/ml ( $\pm 4.2$  SD). Only one woman in this group had a value of 25 ng/ml, which was used as our upper limit of normal ( $\pm 2$  SD).

**Subjects.** Menstrual cycles were classified as abnormal on the basis of the following criteria: amenorrhea ( $>6$  months); oligomenorrhea (cycles  $>45$  days); polymenorrhea (cycles  $<21$  days). Luteal phase abnormalities were classified either as short luteal phase (less than 11 day thermogenic response on basal temperature chart), or luteal phase defect (timed endometrial biopsy on two separate cycles) which showed endometrial development to be "out of phase" by at least 3 days.

Galactorrhea was said to be present if a milky substance was obtained on examination. When the case was doubtful, microscopic examination for fat globules was carried out.

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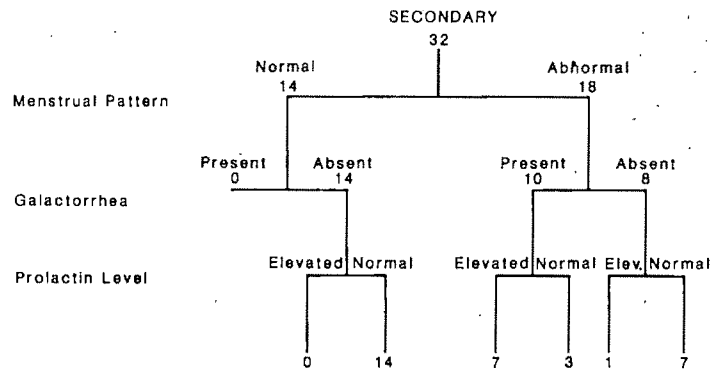


Fig. 1. Secondary infertility, menstrual pattern, galactorrhea, and serum prolactin.

Table I. Patients with hyperprolactinemia and infertility

| Patient               | Prl (ng/ml) | Polytomography | Menstrual pattern | Endometrial biopsy | Galactorrhea |
|-----------------------|-------------|----------------|-------------------|--------------------|--------------|
| Primary infertility   |             |                |                   |                    |              |
| 1.                    | 47.5        | —              | Oligomenorrhea    | ILP                | Yes          |
| 2.                    | 200         | —              | Normal            |                    | Yes          |
| 3.                    | 52.5        |                | Amenorrhea        |                    | No           |
| 4.                    | 200         | +              | Amenorrhea        |                    | Yes          |
| 5.                    | 31.7        |                | Spotting          | Normal             | No           |
| 6.                    | 37          |                | Spotting          | ILP                | No           |
| 7.                    | 80          | —              | Amenorrhea        |                    | No           |
| 8.                    | 27.5        |                | Normal            |                    | Yes          |
| 9.                    | 52.5        | —              | Spotting          | ILP                | Yes          |
| 10.                   | 40.3        |                | Normal            |                    | No           |
| 11.                   | 39.0        |                | Normal            |                    | No           |
| 12.                   | 26.7        |                | Normal            |                    | No           |
| 13.                   | 35.2        |                | Normal            |                    | No           |
| 14.                   | 36.0        |                | Normal            |                    | No           |
| Secondary infertility |             |                |                   |                    |              |
| 15.                   | 68          | +              | Amenorrhea        |                    | Yes          |
| 16.                   | 240         | +              | Amenorrhea        |                    | Yes          |
| 17.                   | 126         | +              | Amenorrhea        |                    | Yes          |
| 18.                   | 77.2        | —              | Amenorrhea        |                    | Yes          |
| 19.                   | 80          | —              | Amenorrhea        |                    | Yes          |
| 20.                   | 31          | —              | Polymenorrhea     | SLP                | Yes          |
| 21.                   | 26          |                | Oligomenorrhea    | Normal             | No           |
| 22.                   | 29          | —              | Oligomenorrhea    |                    | Yes          |

ILP = Inadequate luteal phase. SLP = Short luteal phase

All patients who had galactorrhea were subjected to polytomography of the sella turcica with “2 mm cuts.” Computerized axial tomography (CAT) scan was performed on selected cases on the basis of tomographic findings.

Results

Twenty-two patients had elevated levels of serum prolactin (>25 ng/ml) (19.5%), which was significantly different from the control group ( $\chi^2 = 4.57$ ,  $p < 0.05$ ). The mean level was 71.9 ng/ml, with a range of 26.0 to 240.0 ng/ml. None of these women was hypothyroid or ingesting drugs that might have elevated the serum

prolactin. Of these patients, eight had amenorrhea, three had oligomenorrhea, three had premenstrual spotting, and one had polymenorrhea with a short luteal phase. Timed endometrial biopsy was carried out in the late luteal phase in six of the patients. Two of the three patients with premenstrual spotting showed significant lag in endometrial development consistent with luteal phase inadequacy. This information is summarized in Table I. Because of the small number of subjects, menstrual irregularities and luteal phase abnormalities were combined for analysis.

Twelve of the 22 patients (55%) underwent tomography. Four patients (18%) had pituitary adenomas,

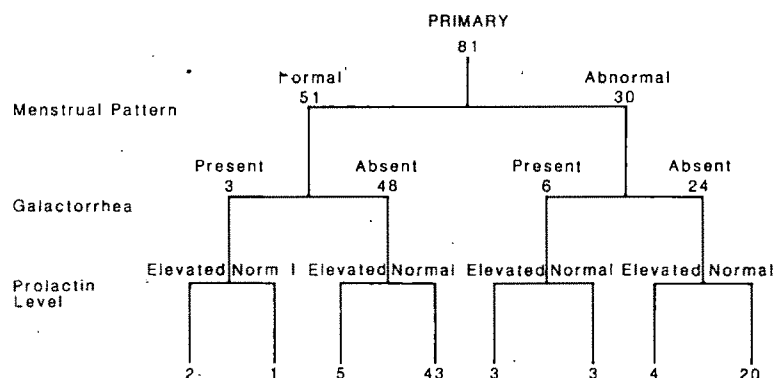


Fig. 2. Primary infertility, menstrual pattern, galactorrhea, and serum prolactin.

but no cause was evident in the other 18. Of these patients, 13 were started on bromocriptine. Live patients had conceived at the time this report was written, and none has yet undergone delivery.

The data were analyzed to show the relationship between type of infertility, menstrual pattern, galactorrhea, and level of serum prolactin. Eight of eight (100%) patients with hyperprolactinemia and secondary infertility had abnormal menses (Fig. 1). Seven of eight (87.5%) patients with secondary infertility and hyperprolactinemia had galactorrhea (Fig. 1). When infertility was combined with abnormal menses or galactorrhea, or both, the incidence of hyperprolactinemia increased. Of particular interest was the finding of a group of five hyperprolactinemic patients with primary infertility, normal menstruation, and no galactorrhea (Fig. 2). This did not reach statistical significance when compared with either secondary infertility ( $\chi^2 = 1.58$ ,  $p < 0.2$ ) or the control population ( $\chi^2 = 1.30$ ,  $p < 0.2$ ).

#### Comment

This study documents a high incidence of hyperprolactinemia in a population of referred infertile

women. Apparently, when a history of menstrual abnormality or a finding of galactorrhea was obtained in these patients, the likelihood of hyperprolactinemia increased. This was especially true of the group with secondary infertility. In that group, no patients with normal menstrual function had hyperprolactinemia.

Several recent studies have reported an association between asymptomatic hyperprolactinemia and infertility, including response to bromocriptine. The significance of hyperprolactinemia in infertile patients who have normal menstrual function but no galactorrhea has not been established. Hyperprolactinemia has been associated with abnormal gonadotrophin<sup>10</sup> and ovarian function<sup>2, 7, 11</sup> which may result in absent or inadequate follicular development and infertility. Beside pituitary tumor, other causes may be previous exposure to oral contraceptives,<sup>12</sup> stress,<sup>5, 13</sup> and abnormal catecholamine metabolism in the central nervous system.<sup>6, 8</sup> Regardless, the incidence of hyperprolactinemia associated with infertility and varying degrees of menstrual dysfunction or galactorrhea demonstrates the usefulness of assaying serum prolactin in the assessment of female infertility.

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# Transvaginal retropubic urethropexy

## The "revised Pereyra procedure": Report of 50 cases

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Fifty patients with uterovaginal prolapse were treated by transvaginal retropubic urethropexy to correct the anatomic defect of their urethrovesical axis. Urodynamic studies were routine in the preoperative assessment. Forty patients had anatomic sphincteric incontinence. Forty-one (82% of the 50 patients treated) were totally continent of urine after a follow-up of 12 to 36 months. The complications and results of this procedure are comparable to those of other abdominal retropubic procedures for urinary incontinence. The suggestion is made that, in the surgical management of symptomatic uterovaginal prolapse, this procedure, which can be easily performed in conjunction with any vaginal operation, may be an alternative to a combined vaginal and abdominal approach. (AM. J. OBSTET. GYNECOL. 139:268, 1981.)

TRANSVAGINAL retropubic urethropexy was performed in 50 patients as an integral step in the management of uterovaginal prolapse by means of the "revised Pereyra procedure"<sup>22</sup>. Reported here are the results with a follow-up period of 12 to 36 months.

### Material and method

Between April, 1977, and May, 1979, 435 patients were seen at the Gynaecologic Urology/Urodynamics Unit at St. Joseph's Hospital for investigation and management of urinary incontinence and/or uterovaginal prolapse. A total of 140 patients received operative treatment during that period. Ninety patients who did not have significant uterovaginal prolapse were treated by a suprapubic procedure. Fifty patients who presented with a degree of symptomatic uterovaginal prolapse severe enough to require a transvaginal approach to the correction of the condition (Grades III and IV uterine prolapse with associated cystourethrocele) were

treated by the indicated vaginal procedure as well as by transvaginal retropubic urethropexy.

All 50 patients with uterovaginal prolapse were studied. They were divided into two groups: Group I consisted of 40 patients who presented with stress urinary incontinence also; and Group II consisted of 10 patients with marked prolapse, including a marked cystocele, but without urinary incontinence.

The age range was 25 to 81 years, with an average of 57.7 years. The average parity was 3.7, with a range of 0 to 11. Only one patient was nulliparous, and 37 patients had a parity of three or more. Thirty-seven of the subjects were postmenopausal. The average duration of symptoms was 3 years, with a range of 2 months to 15 years.

All patients had a thorough history and physical examination. Investigations included voiding metallic bead chain cystourethrography, cystoscopy, and urodynamic studies with the use of a Browne carbon-dioxide cystometer and simultaneous recordings of vesical and urethral pressures.

Of the 40 patients with urinary incontinence, 32 had genuine stress incontinence in which the sphincteric defect was the only demonstrated cause of incontinence. Eight had a "mixed type" of incontinence in which detrusor dyssynergia as well as an anatomic sphincteric defect was demonstrated. The anatomic component, however, was judged to be the predominant cause of urinary incontinence in the preoperative assessments of these eight patients. None of the Group

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I patients had previously received any surgical treatment for urinary incontinence.

Retropubic urethropexy was accomplished by the method described by Pereyra and Lebherz.<sup>22</sup> The associated procedures performed are summarized in Table I.

The procedures, including retropubic urethropexy, were performed by members of the Active Gynaecologic Staff and by the Resident Staff under supervision. At least one of the two authors was present at the operation in all cases. All patients underwent retropubic suction drainage, with Shirley drains\* being placed in the retropubic space, bilaterally, through the anterior vaginal incision prior to closure of the vaginal mucosa. Suprapubic bladder drainage was achieved with use of the Ingram catheter.† Suprapubic catheters were routinely clamped on the fourth postoperative day and removed when three consecutive postvoid residual urines were each 100 ml or less. Bethanechol was used when patients had retention of urine thought to be due to hypofunction of the bladder.

#### Complications

Six patients had cystitis at the time of initial consultation, and all were treated with the appropriate antimicrobial agent. Intraoperative complications included three cases of surgical cystotomy at the time of anterior colporrhaphy; all three were recognized at the time of operation and were successfully repaired. This occurred in one premenopausal woman, 38 years of age, and two postmenopausal women, 51 and 78 years of age; all three patients presented with a total procidentia, two with extreme hypertrophy and thickening of the vaginal mucosa, and one with extreme atrophy of the vaginal mucosa. Thirty-six patients received blood transfusions, ranging from one to five units, with an average of 2.5 units. The institutional transfusion rate for vaginal hysterectomy and repair without retropubic urethropexy is 65.6% (one to six units) and may reflect a liberal attitude toward the use of blood transfusion in vaginal operations. Major immediate postoperative complications are summarized in Table II.

The patient who suffered from the myocardial infarction was known to have cardiac disease preoperatively. Two of the three patients who had wound infection suffered from diabetes mellitus.

Cystitis occurred in 25 patients (50%). The diagnosis was made either with a combination of symptoms, including frequency and dysuria, with or without urgen-

\*Anpro Shirley Wound Drain, H. W. Andorse Products, Inc., Oyster Bay, New York.

†See Contemporary Ob/Gyn 11:1978. The Argyle Co., Sherwood Medical Industries, St. Louis, Missouri.

**Table I.** Associated procedures performed in conjunction with transvaginal retropubic urethropexy

| Procedure                  | No. performed |
|----------------------------|---------------|
| Repair of cystocèle        | 49            |
| Total vaginal hysterectomy | 44            |
| Posterior colporrhaphy     | 44            |
| Repair of enterocele       | 15            |
| The LeFort procedure       | 3             |
| Excision of cervical stump | 1             |
| Repair of urethrocele      | 1             |

**Table II.** Major immediate postoperative complications

| Condition                  | No. of patients |
|----------------------------|-----------------|
| Suprapubic wound infection | 3               |
| Myocardial infarction      | 1               |
| Vaginal vault abscess      | 1               |
| Bacteremia                 | 1               |
| Postoperative fever        | 7               |
| Total                      | 13              |

**Table III.** Microbiologic profile of patients with postoperative cystitis

| Single bacterium                      | No. of patients |
|---------------------------------------|-----------------|
| <i>Escherichia coli</i>               | 7               |
| <i>Streptococcus faecalis</i>         | 2               |
| <i>Proteus mirabilis</i>              | 2               |
| <i>Staphylococcus aureus</i>          | 1               |
| Total                                 | 12              |
| Two bacteria                          |                 |
| <i>E. coli</i> and <i>S. faecalis</i> | 8               |
| Other combinations                    | 5               |
| Total                                 | 13              |
| Total                                 | 25              |

cy, and a positive urine culture, or with a culture growing a single bacterium, with or without symptoms. The microbiologic profile is summarized in Table III.

Postoperatively, spontaneous micturition occurred between 6 and 18 days, with an average of 9.7 days. Results are summarized in Table IV. No patient was discharged with an indwelling urinary catheter. Fourteen patients required orally administered bethanechol chloride to achieve spontaneous voiding.

During the follow-up, the patient who suffered from postoperative myocardial infarction died of cardiac causes 7 months after the operation, having been continent with normal urinary function during that period of time. The other 49 patients were followed for 12 months or longer, including 24 patients who were fol-

**Table IV.** Postoperative interval for onset of spontaneous micturition

| Duration          | No. of patients |
|-------------------|-----------------|
| 8 days or less    | 22              |
| 9 to 11 days      | 15              |
| 12 to 14 days     | 9               |
| 15 days or longer | 4               |
| Total             | 50              |

**Table V.** Follow-up of Group I patients of 12 to 36 months

| Status                            | No. of patients | Percent of total |
|-----------------------------------|-----------------|------------------|
| Cured                             | 32              | 82.0             |
| Improved                          | 3               | 7.7              |
| Failure of procedure              | 4               | 10.3             |
| Lost to follow-up (medical death) | 1               |                  |
| Total                             | 40              | 100.0            |

lowed for 2 years or longer. Although the follow-up period in one half of the patients has been relatively short (12 months), the preliminary results to date suggest that the procedure of transvaginal retropubic urethropexy is of value in the surgical management of advanced uterovaginal prolapse and anatomic urinary incontinence. Patients in Group I were considered to be cured when they no longer suffered from urinary incontinence, and to be improved when they continued to suffer from urinary incontinence intermittently but with less severity and frequency; the operation was considered to have failed when the patient became incontinent of urine at any time in the follow-up period. None of the 10 patients in Group II became incontinent as a result of the operation. The results are presented in Table V.

One of the four failed and two of the three improved patients were diagnosed as having "mixed type" incontinence preoperatively. One of the failed patients was continent until the Prolene sutures were inadvertently removed when she underwent repair of an inguinal hernia 4 months after the retropubic urethropexy. The remainder of the patients, two in the failed group and one in the improved group, all had good anatomic support of the urethrovesical neck postoperatively. Urodynamic reassessment disclosed no definite cause for the failure.

Late postoperative complications are summarized in Table VI. Only the symptomatic conditions were subsequently corrected surgically. The one case of recurrent rectocele occurred with a recurrent enterocele and was repaired in conjunction with the enterocele.

Results in the patients of Group I were analyzed ac-

**Table VI.** Long-term postoperative complications in 49 patients

| Condition              | No. of patients | Surgically corrected |
|------------------------|-----------------|----------------------|
| Recurrent cystocele    | 2               | 0                    |
| Recurrent rectocele    | 3               | 1                    |
| Enterocele             | 4               | 2                    |
| Recurrent enterocele   | 2               | 1                    |
| Vaginal vault prolapse | 3               | 1                    |
| Total                  | 14              | 5                    |

**Table VII.** Results of the Pereyra procedure

| Author                                    | No. of patients | Cure rate (%)             | Follow-up (mo.) |
|-------------------------------------------|-----------------|---------------------------|-----------------|
| Pereyra, <sup>20</sup> 1959               | 31              | 90                        | 14              |
| Harer and Gunther, <sup>10</sup> 1965     | 30              | 87                        | 2-36            |
| Pereyra and Leberherz, <sup>21</sup> 1967 | 210             | 94.3                      | 12-24           |
| Crist et al., <sup>7</sup> 1969           | 172             | 91.9                      | 12-89           |
| Crist et al., <sup>7</sup> 1969           | 56              | 54                        | 12-41           |
| Backer and Probst, <sup>1</sup> 1971      | 80              | 85                        | 6-42            |
| Kursh et al., <sup>16</sup> 1972          | 25              | 64                        | 12              |
| Portnuff and Bal-lon, <sup>24</sup> 1973  | 40              | 92.5                      | 12-60           |
| Litvak and McRoberts, <sup>17</sup> 1974  | 35              | 88.6                      | 36-60           |
| Backer and Probst, <sup>2</sup> 1976      | 200             | 82—Cured<br>10.5—Improved | 6-108           |

cording to the length of the follow-up period. Of the 39 patients, 21 were followed for 2 years or longer. There were two failures in this subgroup, for a failure rate of 9.5%. Eighteen patients were followed for 12 to 24 months, with two failures, for a rate of 11%.

### Comment

Kelly and Dumm<sup>15</sup> first introduced insertion of mattress sutures at the vesical neck to cure women with urinary incontinence "without manifest injury to the bladder," a procedure which now bears Kelly's name. Kelly claimed a success rate of 80%. However, subsequent workers reported a success rate of only 48% to 63% with the use of a vaginal approach.<sup>3, 13, 18, 25</sup> Marshall and associates<sup>19</sup> described the suprapubic approach for correction of urinary incontinence in 1949, and the Burch<sup>5</sup> modification of that procedure was described in 1961. Since then, the suprapubic approach, as described by Green,<sup>8</sup> who modified Bailey's<sup>4</sup> classification, has become increasingly popular as a primary treatment for patients with a Type II urethrovesical defect. The philosophy of "vaginal repair first" with reservation of the abdominal retropubic approach for failures was subsequently challenged.<sup>12, 27</sup>

In 1959, Pereyra<sup>20</sup> introduced a surgical procedure



for cure of urinary incontinence by placing sutures in the paraurethral tissue and anchoring them to the abdominal wall, thereby supporting the urethrovesical neck. This was achieved by using a ligature carrier. However, the procedure was performed blindly and perforation of the bladder was a major complication, as reported by Goldstein.<sup>9</sup> Results reported in the literature are summarized in Table VII.

A revised procedure was subsequently described by Pereyra,<sup>22</sup> who reported 29 patients with a follow-up period of 24 months or longer. Of these 29 patients, 27 were symptomatically and objectively cured and two were improved.<sup>23</sup>

In the patients of this study, vaginal hysterectomy and/or vaginal plastic repair were judged to be necessary to correct the symptomatic uterovaginal prolapse. Furthermore, a retropubic procedure was thought to be necessary to correct the severity of the anatomic deficit of the urethrovesical axis associated with the stress urinary incontinence in the 40 patients in Group I.

In the management of the 10 patients in Group II, consideration was given to a case described by Marshall and associates<sup>19</sup> of a woman who "became" incontinent after an anterior colporrhaphy, as well as to observations by Jeffcoate and Roberts<sup>14</sup> that, in patients who suffer from a prolapse wherein the bladder and urethra descend together, the urethrovesical relationship remains intact, so that the patient remains continent. However, the posterior urethrovesical angle could be obliterated by an anterior colporrhaphy with tight plication of tissue around the urethrovesical neck and the correction of the cystocele. As a result, urinary incontinence can occur in a number of these patients postoperatively. Therefore, a retropubic procedure was thought to be necessary in conjunction with the vaginal procedures to correct the prolapse in the patients in Group II and return the urethrovesical neck to an intra-abdominal position, thereby minimizing the possibility of the occurrence of urinary incontinence postoperatively. The "modified Pereyra procedure" was chosen as the appropriate retropubic urethropexy and was performed in conjunction with the other indicated vaginal procedures.

Enterocoele formation is a recognized long-term complication of retropubic urethropexy procedures. Incidences of 7.6% and 17% were reported by Burch<sup>6</sup> and Stanton and associates,<sup>26</sup> respectively. In this series, recurrent enterocoele occurred in two of the 15 patients (13.3%). Of the 35 patients who did not have an enterocoele in the initial presentation, four (11.7%) developed an enterocoele in the follow-up period. All cases of enterocoele were detected between 6 and 12 months postoperatively.

The failure rate among patients in Group I was 10.3%; but if the one case in which the sutures were removed inadvertently is allowed for, a corrected failure rate of 7.7% was achieved. The importance of accurate preoperative diagnosis must be stressed. Two of the improved patients and one of the failed patients had the "mixed type" of incontinence according to the initial preoperative assessment. In retrospect, the judgment that the anatomic component was predominant was in error, and the patient continued to suffer from intermittent loss of urine because of preoperatively identified detrusor dyssynergia, the persistence of which was confirmed by postoperative urodynamic reevaluation. Failures in this study were all identified within 12 months of operation. This may suggest that, if a patient is cured at 12 months follow-up, the likelihood is that she will remain continent. However, long-term follow-up for a minimum of 5 years is still considered to be essential.<sup>11</sup>

The data presented here are insufficient to allow the drawing of any concrete conclusions. The results and complications of this procedure are comparable to those of other abdominal retropubic procedures.<sup>6, 26</sup> Therefore, the suggestion is made that, when surgical intervention is indicated, transvaginal retropubic urethropexy may have a part in the management of patients who have uterovaginal prolapse, with or without urinary incontinence. The procedure can be performed in conjunction with any vaginal procedures that require only a small suprapubic incision. Thus, the use of a combined vaginal and abdominal approach may be avoided in selected cases.

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# Urodynamic studies in female patients with multiple sclerosis

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Patients with multiple sclerosis may have two fundamental, related problems in the lower urinary tract, i.e., urinary retention and/or urinary incontinence. Forty-six patients were assessed by urodynamic testing. Evidence of detrusor overactivity was seen in 40 patients (86.9%), elevated resting urethral pressures in 39 patients (84.8%), a positive bethanechol chloride (Urecholine) stress test in 24 patients (63.2%), and a significant residual urine in 26 patients (56.5%). Data were analyzed in an attempt to explain retention and incontinence in these patients. (AM. J. OBSTET. GYNECOL. 139:273, 1981.)

MULTIPLE SCLEROSIS is a relatively common neurologic disease in Western Canada (approximately 130 cases per 100,000 population). Problems of the lower urinary tract are common in female patients with multiple sclerosis. In a review of 297 patients with multiple sclerosis, Miller and associates<sup>7</sup> found that 2% presented with a disturbance of micturition, and that 12% had micturition difficulties among the complaints at initial presentation. Those authors also reported that bladder problems eventually developed in 78% of the same group. In a review, by Kurtzke,<sup>6</sup> of 1,623 patients with multiple sclerosis, one third of them and one half of all hospitalized patients had bladder problems.

From a clinical standpoint, it seemed to us that patients with multiple sclerosis and related difficulties in the lower urinary tract had one or both of two problems. Urinary retention, varying from difficulty in emptying the bladder to total inability to void, was a major problem (very often with associated cystitis) for some patients, whereas others seemed to be more troubled by symptoms suggestive of detrusor overactivity, such as frequency, urgency, urgency or stress incontinence, nocturia, and bladder spasm.

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Although Summers<sup>9</sup> and Bradley<sup>4</sup> have reported the results of cystometric and electromyographic studies in a large number of patients with multiple sclerosis, our unit decided to study, in a prospective manner, female patients with multiple sclerosis, by means of a somewhat different urodynamic profile in order to further delineate the nature of the bladder problems and to determine whether urodynamic studies could or could not be used as an adjunct in diagnosing multiple sclerosis.

The senior author (R. P. B.) wishes to note for the clarification of the reader that he recognizes three types of urinary stress incontinence: an anatomic or pressure equalization type of stress incontinence; a detrusor overactivity type of stress incontinence which is synonymous with "unstable bladder" or detrusor dys-synergia; and a combined type of stress incontinence involving detrusor overactivity and pressure equalization.<sup>2</sup>

Forty-six consecutive female patients with symptoms relative to the lower urinary tract, with acute or chronic multiple sclerosis, were studied by means of a urodynamic profile. The patients with an acute attack of multiple sclerosis had acute optic neuritis plus other symptoms or signs of disseminated demyelination, whereas patients were considered to have chronic multiple sclerosis on the basis of the criteria of Schumacher and associates.<sup>8</sup> The age of onset, duration of disease, and functional disability, according to the Kurtzke scale,<sup>5</sup> were recorded for each patient. All patients had some ambulatory capability.

The urodynamic assessment consisted of: measurement of bladder capacity (volume producing urge);

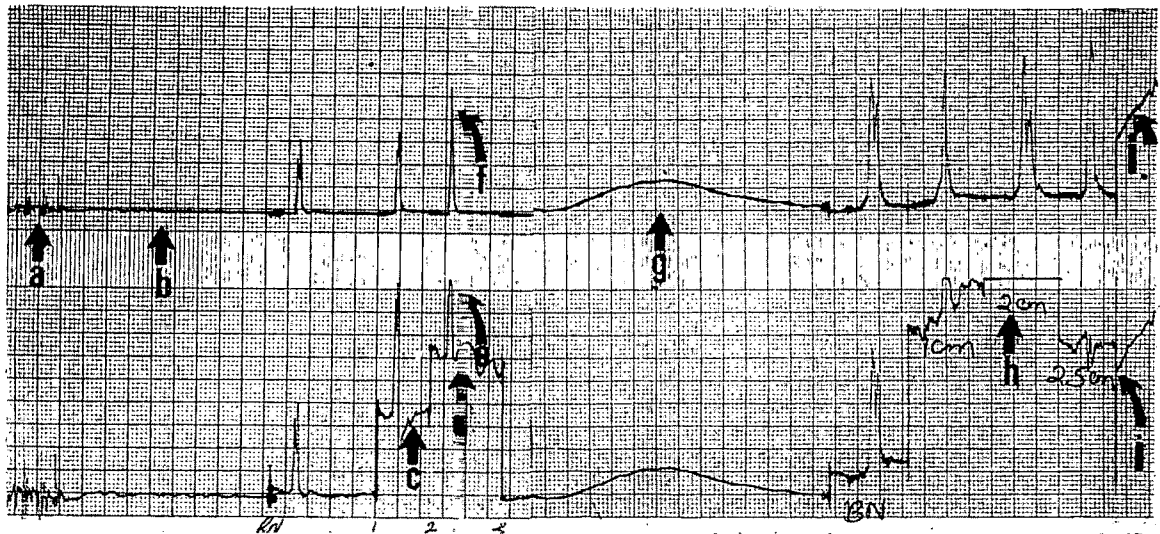


**Fig. 1.** Simultaneous intraurethral and intravesical pressures (normal and with multiple sclerosis). The intravesical pressure is at the top, and the intraurethral pressure is at the bottom. Each demarcation represents 2 mm Hg. Paper speed is 2 cm/sec. The tracing to the left is of a normal continent woman, and that to the right is of a patient with multiple sclerosis. Heel bounce (a) does not trigger detrusor contraction since baseline pressure stays constant (b). Arrows c and d show normal urethral pressures in the range of 40 to 60 mm Hg. Urethral kinking (e) with cough offsets cough pressure spike in bladder (f), and continence is maintained. Arrow h points to extremely high urethral pressure in patient with multiple sclerosis. Detrusor contraction wave (g) which occurred without any stress indicates detrusor overactivity as do arrows (i) which point to rise in baseline bladder pressure which occurred in change from supine to standing position. (Both balloons in bladder at this point.) The detrusor overactivity produced incontinence despite high urethral pressures.

**Table I.** Results of seven urodynamic assessments in 46 patients

|                                          | Normal | Increased | Decreased | Positive | Negative | Total No. of patients |
|------------------------------------------|--------|-----------|-----------|----------|----------|-----------------------|
| Bladder capacity                         | 46     | 2         | 1         | —        | —        | 46                    |
| Residual urine                           | 26     | 26        | —         | —        | —        | 46                    |
| Urethral pressure                        | 3      | 39        | 4*        | —        | —        | 46                    |
| Baseline bladder pressure                | 11     | 35        | —         | —        | —        | 46                    |
| Detrusor contraction waves               | —      | —         | —         | 38       | 8        | 46                    |
| Demonstrable stress urinary incontinence | —      | —         | —         | 16       | 30       | 46                    |
| Bethanechol chloride stress test         | —      | —         | —         | 24       | 14       | 38                    |

\*All four patients were stress incontinent.

measurement of residual urine; simultaneous intraurethral and intravesical pressure studies with the bladder stressed to the point of urge, with the use of a slow transurethral infusion of normal saline solution warmed to body temperature, in order to measure urethral pressures<sup>1</sup> and to detect detrusor overactivity<sup>2,3</sup>; a test for stress incontinence with coughing and heel bouncing; and a bethanechol chloride (Urecholine) stress test performed with 100 cc of fluid in the bladder, using a continuous intravesical pressure recording for 30 minutes after a subcutaneous injection of 2.5 mg.

A bladder capacity of 200 to 600 cc was considered to be normal. Less than 30 cc was considered to be a nor-

mal residual urine. A normal intraurethral pressure was considered to be in the range of 40 to 60 mm Hg for the maximal pressure encountered in any centimeter of the urethra. Detrusor overactivity was diagnosed whenever there was a rise in baseline bladder pressure of more than 10 mm Hg or detrusor contraction waves with an amplitude greater than 4 mm Hg. The bethanechol chloride stress test was considered to be positive if there was a detrusor contraction wave or a rise in baseline bladder pressure of more than 15 mm Hg.

Urodynamic tests were performed in all 46 patients, except eight who did not have a bethanechol chloride stress test.



## Results

The duration of multiple sclerosis varied from less than 1 year to 32 years, with a mean of 8.0 years. The Kurtzke scale ranged from 1 to 7 (mean, 3.0). The results of seven urodynamic assessments are indicated in Table I. Perhaps the most striking urodynamic finding was the marked elevation in resting urethral pressures seen in 84.8% of the patients, often in the range of 70 to 90 mm Hg (Fig. 1). Bradley<sup>4</sup> previously emphasized this finding. One important observation not indicated in Table I is that all but six patients demonstrated evidence of detrusor overactivity in the form of detrusor contraction wave(s) and/or elevation of baseline bladder pressure. The data on duration of disease, Kurtzke scale and in Table I, was subjected to chi-square, Pearson correlation, and Spearman correlation analysis, depending on the variable type.

Statistical analysis is shown in Table II.

## Comment

The duration of disease statistically correlated, understandably, with the Kurtzke disability score and also with increased residual urine and a positive bethanechol chloride stress test.

An increased residual urine correlated with the duration of disease, the Kurtzke disability score, and a positive bethanechol chloride stress test, but *not* with elevated urethral pressure. The foregoing suggests that urinary retention is not due to increased urethral resistance, but rather to a myoneural dysfunction of the detrusor muscle.

It would seem that the urethra can be affected by a spastic upper motor neurone-type lesion, with multiple sclerosis causing abnormally high resting intraurethral pressures. This lesion occurred in 39 of 46 patients and was unrelated to duration of disease and Kurtzke disability. The fact that abnormally high resting urethral pressures (resistance) correlated with continence suggests that the high urethral pressures may have a compensatory effect in maintaining continence in the face of detrusor overactivity which was so common (86.9% of cases) either in the form of detrusor contraction waves or elevated baseline bladder pressures. The high urethral pressures are probably caused by spasm of the striated and/or circular smooth muscle components of the urethra.

The urethra can also be affected by a flaccid lower motor neurone-type lesion, with multiple sclerosis, which was associated with urinary stress incontinence in all four of the cases associated with abnormally low urethral pressures. The urethral pressures in these cases were much lower than usually seen in patients with anatomic stress incontinence (low urethral pres-

Table II. Statistical analysis

|                                                                                                                                          |
|------------------------------------------------------------------------------------------------------------------------------------------|
| Significant correlation between duration of disease and Kurtzke disability scale ( $p < 0.001$ )                                         |
| Significant correlation between duration of disease and a positive bethanechol chloride stress test ( $p < 0.05$ )                       |
| Significant correlation between duration of disease and increased residual urine ( $p < 0.05$ )                                          |
| Significant correlation between the Kurtzke disability scale and significant residual urine ( $p < 0.05$ )                               |
| Significant correlation between a positive bethanechol chloride stress test and significant residual urine ( $p < 0.05$ )                |
| Significant correlation between detrusor contraction wave(s) and elevated baseline bladder pressure ( $p < 0.01$ )                       |
| Significant correlation between detrusor contraction wave(s) and stress incontinence ( $p < 0.005$ )                                     |
| Significant correlation between elevated baseline bladder pressure and stress incontinence ( $p < 0.01$ )                                |
| Significant correlation between elevated urethral pressures and <i>absence</i> of stress incontinence ( $p < 0.005$ )                    |
| Significant correlation between a positive bethanechol chloride stress test and detrusor contraction wave(s) ( $p < 0.025$ )             |
| No correlation between a positive bethanechol chloride stress test and elevated baseline bladder pressure                                |
| No correlation between elevated urethral pressures and elevated baseline bladder pressure(s)                                             |
| No correlation between a positive bethanechol chloride stress test and urinary stress incontinence                                       |
| No correlation between a positive bethanechol chloride stress test and elevated urethral pressures                                       |
| No correlation between elevated urethral pressures and duration of disease, Kurtzke score, nor with increased baseline bladder pressures |

sure type<sup>1</sup>). Resistance in the urethra in these cases was almost nonexistent. The flaccid urethra, also, did not correlate with duration or severity of disease.

Urinary stress incontinence correlated with detrusor contraction wave(s), elevated baseline bladder pressure, and decreased urethral pressures. The fundamental cause of stress incontinence in these patients would seem to be detrusor overactivity and, less frequently, decreased urethral (pressures) resistance. There was no correlation between incontinence and a positive bethanechol chloride stress test.

It would seem that the urethra and bladder were separate entities in relation to affection by this neurologic disease. Detrusor contraction waves and elevated baseline bladder pressures correlated with each other, and detrusor contraction waves correlated with a positive bethanechol chloride stress test. The lack of correlation between a positive bethanechol chloride stress test and elevated baseline bladder pressures was an inexplicable surprise to us. There was no correlation between high resting intraurethral pressures and elevated baseline bladder pressures, nor with a positive bethanechol chloride stress test, thus suggesting that spasm of the urethra did not correlate with spasm of the bladder, although there was some correlation be-

tween high urethral pressures and detrusor contraction waves.

The urodynamic profile typical of patients with multiple sclerosis would seem to be the following: increased resting urethral pressures, increased residual urine, evidence of detrusor overactivity as adjudged by de-

trusor contraction wave(s) and/or elevated baseline bladder pressure, urinary stress incontinence, and a positive bethanechol chloride stress test.

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# Acute adolescent menorrhagia

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Acute menorrhagia in adolescence is a much underestimated clinical problem; often requiring urgent medical intervention. In a 9-year case review between January, 1971, and January, 1980, we looked at all admissions to a children's hospital for acute menorrhagia, where genital tract pathology had been excluded. A primary coagulation disorder was found in almost 20% of these 59 patients. One quarter of those with severe menorrhagia (hemoglobin less than 10 gm/100 ml), one third of those requiring transfusion, and one half of those presenting at menarche had such an underlying disorder. Therefore, proper screening and therapy are essential in all girls with menorrhagia. Conventional methods of hormonal control are only partially effective in these special cases. (AM. J. OBSTET. GYNECOL. 139:277, 1981.)

MENSTRUAL IRREGULARITIES, including menorrhagia, are common gynecologic problems in adolescents and are usually easily managed in an outpatient setting. Acute menorrhagia, requiring hospitalization and urgent medical management, is a less common but much underestimated clinical problem in terms of the dramatic presentation of these patients.<sup>1</sup> For example, it is not unusual to be faced with a pale, frightened teenager in profound hypovolemic shock with a hemoglobin of 6 gm/100 ml as a result of menorrhagia with her first or second menstrual period. The majority of these cases are attributed to dysfunctional uterine bleeding or simple anovulation. The purpose of this paper is to emphasize that a significant proportion of adolescents with acute menorrhagia have an underlying coagulation disorder which requires elucidation and specific therapy.

## Material and methods

Our experience with these patients prompted a review of all admissions to the Hospital for Sick Children for acute menorrhagia between January, 1971, and January, 1980. During this time, all admissions were to

the gynecologic service under the same two staff consultants. Therefore, despite minor individual variations, there was essential uniformity in diagnostic screening and management.

The following factors were assessed: the incidence of coagulation disorders in the etiology of menorrhagia (as opposed to purely anovulatory bleeding), the hemoglobin on admission, the incidence of transfusion, the role of dilatation and curettage, and whether the admitting episode occurred at menarche or in later "gynecologic years." We also devised a practical approach to achieving hormonal hemostasis in these patients.

## Results

A total of 83 new cases of acute menorrhagia occurred during the 9-year study period. Twenty-four cases were excluded on the basis that menorrhagia was not the primary admitting diagnosis. The remaining 59 cases were suitable for detailed review.

Of these 59, 44 or 74% were diagnosed as having dysfunctional uterine bleeding by a technique of exclusion, and 11 or 19% were diagnosed as having a coagulation disorder leading to excessive uterine blood loss. The coagulation disorders comprised four cases of idiopathic thrombocytopenic purpura, three cases of von Willebrand's disease, two cases of the rare Glanzmann's disease (which is congenital thrombasthenia, a primary qualitative platelet defect)<sup>2-4</sup>, one case of thalassemia major, and one case of Fanconi's anemia, the last two both presenting with thrombocytopenia. Four cases or 7% were found to have other major contributing factors to menorrhagia, including one 12-

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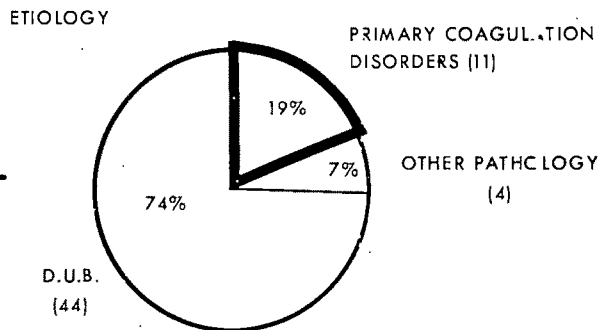


Fig. 1. The etiology of acute adolescent menorrhagia in 59 cases. The figures in parentheses represent the number of cases in each group.

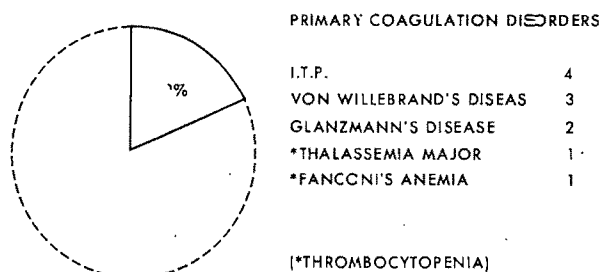


Fig. 2. Primary underlying coagulation disorders in 11 cases of acute adolescent menorrhagia.

year-old girl with endometrial stromal sarcoma, subsequently diagnosed at curettage (Figs. 1 and 2).

Forty-nine percent of the patients presented with severe menorrhagia, as defined by a hemoglobin of less than 10 gm/100 ml on admission. In terms of etiology the following were found: 62% had dysfunctional uterine bleeding; 28%, or one in four, had an underlying coagulation disorder; and 10% had other pathology. The mean initial hemoglobin was 7.9 gm/100 ml.

Of the total admissions, 26 or 44% required transfusion of blood or blood components, often for hypovolemic shock, and 35% or one in three of these had a coagulation disorder.

All 59 patients were treated by means of initial hormonal hemostasis. Curettage was additionally performed in 34%. This liberal use of surgical treatment occurred in the earlier years of the study, prior to the general acceptance of primary medical management. The yield of significant abnormality was very low (one case).

The number of patients presenting with life-threatening uterine blood loss during their first menstrual period was nine, or 15% of the total, but this included five of the 11 patients with coagulation disorders, or 45% of this group of girls, and illustrates the importance of excluding a hematologic problem whenever

menorrhagia occurs at menarche. (In one case of von Willebrand's disease, the diagnosis was initially made because of the severity of the menorrhagia dramatizing the onset of menarche.)

### Comment

The conclusions that can be drawn from this long-range experience with acute adolescent menorrhagia are that an underlying coagulation disorder can be found in one of five girls requiring hospitalization, one of four with severe menorrhagia and a hemoglobin less than 10 gm/100 ml, one of three needing transfusion, and one of two presenting at menarche.

In other words, there are certain predictive factors that lead to a high index of suspicion of a hematologic problem in the etiology of acute menorrhagia in adolescents. Therefore, it is recommended that, in addition to a careful medical history to elicit symptoms of easy bruising, epistaxis, and gingival bleeding, together with the family history and a thorough physical examination, a blood smear and coagulation screen be performed routinely. The latter should include the prothrombin time, partial thromboplastin time, platelet count, and bleeding time and will effectively rule out all but the rarest of the hemorrhagic diatheses. These tests should be performed prior to transfusion or the initiation of hormonal therapy. It is also suggested that thyroid function tests (thyroxine, triiodothyronine resin uptake, and thyroid-stimulating hormone), urinalysis, and a screening blood glucose level be done to complete the laboratory evaluation in the adolescent age group.

**Management protocol.** The management protocol for adolescents with acute menorrhagia is individualized according to the clinical presentation. If bleeding is not heavy and the hemoglobin is greater than 10 gm/100 ml, watchful expectancy is a reasonable approach, with the anticipation that the majority of patients will spontaneously convert to normal menstrual cycles within 1 to 2 years. The decision to preempt cycles with a combined estrogen-progestin preparation is individual. Oral iron replacement is, of course, required. In the meantime, careful follow-up is undertaken to rule out chronic occult illness, to assess the timing and duration of menses, and to treat any relapse that may occur. Persistent anovulation will necessitate full endocrinologic evaluation. Those patients whose dysfunctional bleeding episodes were preceded by normal menses have a more favorable prognosis.

In this series of patients, all were admitted to the hospital because of an acute episode of bleeding which was judged to require emergency management, although severe anemia (hemoglobin less than 10 gm/



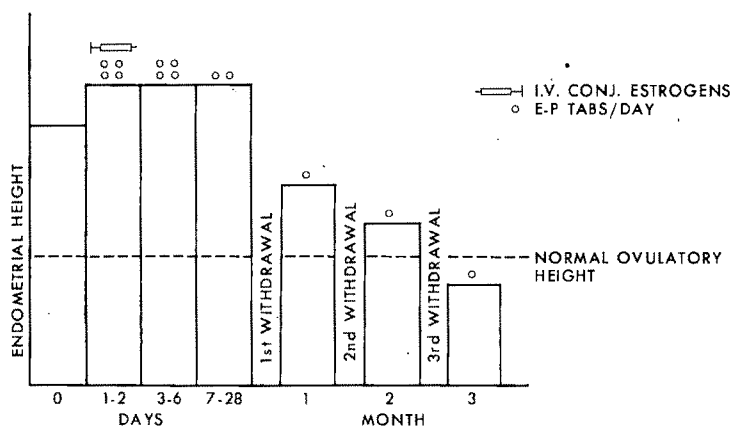


Fig. 3. A schematic presentation of hormonal hemostasis in managing acute adolescent menorrhagia.

100 ml on admission) was not present in all cases. Those patients with a known coagulation disorder were treated primarily with appropriate replacement therapy, i.e., transfusion of blood, cryoprecipitate, or platelet concentrates. Corticosteroids were also used in those cases of idiopathic thrombocytopenic purpura in which they were appropriate. The administration of sex steroids was adjunctive therapy in these patients.

Our approach to achieving initial hormonal hemostasis in any case of acute menorrhagia, particularly when bleeding has been prolonged or is very heavy, is to administer high-dose conjugated estrogens,\* 40 mg intravenously every 4 hours for a maximum of six doses. Concurrently, a highly progestational combined estrogen-progestin oral preparation (containing 5 mg of progestin†) is given, with antiemetics if necessary. An initial loading dose of two tablets is followed by one tablet four times daily. This dosage can be gradually tapered over the following month according to the response. A patient who requires maximal doses of conjugated estrogens will also need additional progestin (e.g., norethindrone acetate, ‡ 5 mg four times a day) to counteract the potent estrogenic effects. It is unnecessary to continue parenteral estrogen therapy beyond 24 hours (or very occasionally 48 hours), and uncontrolled bleeding beyond this time is an indication for re-evaluation by examination with the patient under anesthesia and diagnostic dilatation and curettage. It is important to realize that neither high-dose estrogen nor progestin will work effectively alone.

Following this intense hormonal therapy, three months of cyclic therapy with conventional combined oral contraceptive pills is undertaken, with the expectations that the withdrawal flow will be reasonable and

regular and that each successive cycle will be accompanied by progressive reduction in endometrial height (Fig. 3).

After three preempted cycles, the patient is reassessed and a decision is made to allow her to menstruate without medication. If the anovulatory pattern recurs, a progestin such as medroxyprogesterone acetate\* (10 mg daily for 5 days every 40 days) is an excellent cycle regulator. It allows spontaneous menses to occur between treatment cycles and it prevents long-term, unopposed estrogen stimulation of the endometrium.

The long-term prognosis of the patients in this study has yet to be assessed. Many have been lost to further follow-up 6 to 12 months after their admission to hospital. This may suggest that their problem has resolved, but their prognosis remains, at best, guarded. It has been shown that about 5% of patients continue to have severe episodes of anovulatory dysfunctional bleeding, and these patients merit endocrinologic investigation for the cause of chronic anovulation.<sup>5</sup>

In general, patients with acute adolescent menorrhagia constitute a high-risk group in which there is an increased incidence of anemia, transfusions, operations, and subsequent infertility, spontaneous abortions, and impaired reproductive potential. Of even greater significance is the risk of endometrial adenomatous hyperplasia and frank carcinoma in later life.<sup>5</sup>

The importance of long-term follow-up of these girls is shown by the fact that 2 years after the onset of the presenting episode, bleeding problems continue in almost 60%; at 4 years, in 50%; and at 10 years and over, in 30% to 40%.<sup>6</sup> The worst prognosis is found in those with menorrhagia at the time of menarche.

In the girls with underlying coagulation disorders,

\*Premarin, Ayerst Labs.

†Ortho-Novum, Ortho Pharmaceuticals.

‡Norlutate, Parke, Davis & Co.

\*Provera, The Upjohn Co.

severe bleeding episodes may occur on a hormonal basis or because of reactivation or poor control of the basic hematologic condition. We have found it necessary, on occasion, to induce prolonged amenorrhea by

suppression of menses in some patients with "unstable" hematologic diseases.<sup>7</sup> These girls remain a therapeutic challenge, best managed by the combined efforts of both a pediatric hematologist and a gynecologist.

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# Management of congenital absence of the vagina

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The management of congenital absence of the vagina is facilitated where there is knowledge and understanding of any associated genetic, metabolic, and morphologic anomalies. Although the McIndoe split-thickness graft operation was the most commonly used method (15 patients), other surgical and nonsurgical alternatives were selectively utilized. In 10 women where a functioning uterus was present, laparotomy was necessary in six because of obstruction to uterine drainage. In the choice of treatment method to establish a functioning vagina, individualization is important, but certain guiding principles found to be useful are discussed. (AM. J. OBSTET. GYNECOL. 139:281, 1981.)

CONGENITAL ABSENCE of the vagina is defined as complete or partial failure of vaginal development. To be of clinical significance, the anomaly must cause or have the potential to cause obstruction to coitus and/or to uterine drainage. Women with this condition constitute a heterogeneous group with a high incidence of genetic and metabolic disorders and associated anomalies of the müllerian duct as well as other body systems.

Three widely differing methods of achieving a functioning vagina have been utilized in the series under review. Operative methods include the application of a split-thickness graft to a preformed vaginal space as described by McIndoe and Bannister<sup>6</sup> and the creation of a vulvovaginal pouch as described by Williams.<sup>11</sup> The nonsurgical alternative has been the application of the principle of repeated directed pressure by means of a plastic dilator as described by Frank.<sup>1</sup> The factors relative to the choice of treatment method and the results of treatment have been reviewed.

### Patients and methods

From 1966 to 1980, 45 patients were assessed and/or treated at the Vancouver General Hospital. They have been assigned where possible, into specific groups (Table I), which has permitted recognition of the relative incidence of the types represented and has facilitated the evaluation of therapeutic alternatives based

Table I. Congenital absence of the vagina

|                                                                                        | No. |
|----------------------------------------------------------------------------------------|-----|
| <i>Group 1: Absence of a functioning uterus:</i>                                       |     |
| Uterovaginal agenesis<br>(Rokitansky-Kuster-Hauser syndrome)                           | 19  |
| Feminizing male pseudohermaphroditism<br>(testicular feminization)                     | 11  |
| Masculinizing male pseudohermaphroditism<br>(pseudovaginal perineoscrotal hypospadias) | 5   |
| <i>Group 2: Functioning uterus</i>                                                     |     |
| Vaginal atresia                                                                        | 8   |
| Urogenital sinus with anorectal<br>communication                                       | 2   |

Table II. Vaginal depth at time of presentation

|                                                                  | <1 cm | 1-3 cm | 3-5 cm | >5 cm |
|------------------------------------------------------------------|-------|--------|--------|-------|
| Uterovaginal agenesis<br>(19 patients)                           | 13    | 4      | 2      | 0     |
| Feminizing male<br>pseudohermaphroditism<br>(11 patients)        | 0     | 3      | 7      | 1     |
| Masculinizing male<br>pseudohermaphroditism<br>(5 patients)      | 3     | 2      | 0      | 0     |
| Vaginal atresia<br>(8 patients)                                  | 4     | 3      | 1      | 0     |
| Urogenital sinus with<br>anorectal communication<br>(2 patients) | 2     | 0      | 0      | 0     |

on the nature and origin of the defect. The depth of the vagina at the time of initial presentation (Table II) can be used as a guide to help in identification of the clinical syndrome of which the vaginal anomaly is one part, as well as being a basic consideration in the selection of the mode of management.

The treatment methods used in the 35 patients in whom a functioning uterus was not present (group 1)

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**Table III.** Treatment summary in group 1 with no uterus

|                                                       | <i>Split thickness graft to vaginal space (McIndoe)</i> | <i>Vulvo-vaginoplasty (Williams)</i> | <i>Pressure (Frank)</i> | <i>No treatment or treatment pending</i> |
|-------------------------------------------------------|---------------------------------------------------------|--------------------------------------|-------------------------|------------------------------------------|
| Uterovaginal agenesis (19 patients)                   | 10                                                      | 2                                    | 2                       | 5                                        |
| Feminizing male pseudohermaphroditism (11 patients)   | 3                                                       | 0                                    | 2                       | 6                                        |
| Masculinizing male pseudohermaphroditism (5 patients) | 2                                                       | 0                                    | 2                       | 1                                        |

are recorded in Table III. No direct treatment to alter the vagina has yet been carried out in six prepubertal children and it has been deferred in six patients with a vaginal depth of 4 cm or greater, in whom coitus has proved to be satisfactory, or in whom coital difficulties are not anticipated.

Therapeutic methods in the 10 patients in whom the vaginal anomaly coexisted with a functioning uterus (group 2) were more varied and individualized. In addition to providing for a functioning vagina for coitus, a therapeutic responsibility was to manage a real or potential obstruction to uterine secretions. Six patients underwent laparotomy for assessment of a developing abdominal mass. Surgery to provide for coital function has been performed in three patients. The atretic segment was excised or incised in two patients without a graft, and in one patient a split-thickness graft was used to cover the excised atretic segment as in a McIndoe procedure. No treatment was required in one patient, who was having satisfactory coitus with a vaginal depth of 4 cm. In the remaining four prepubertal girls, a definitive approach to the creation of a functioning vagina has not yet been undertaken. In one of the two patients presenting with a urogenital sinus and an anorectal communication, a vulvovaginoplasty to provide a coital pouch by the Williams technique was performed.

### Results

In the 15 patients treated by the split-thickness graft to the vaginal space (McIndoe operation), the average hospital stay was 16 days. There was no serious postoperative morbidity. No significant complications and no fistulas developed. One woman presented 4 years after the operation with vaginal bleeding and was found to have an epithelium-lined space beneath the anterior vaginal wall, created when the skin graft had lifted from its attachment. With the excision of the central portion of the anterior wall graft, the underlying space was brought into full communication with the remainder of the graft-lined vagina and satisfactory coital

function continued. On follow-up ranging from 6 months to 10 years, 10 of the 15 reported regular intercourse without difficulties. In two without regular sexual partners, the vagina was noted to be somewhat reduced in length but coital function was possible. Three women were lost to follow-up after the initial 6 months.

Follow-up on the three patients who were managed by the Williams vulvovaginoplasty has been short. One woman reported satisfactory coital function prior to being lost to follow-up at approximately 7 months. Although coitus is reported to be satisfactory in the remaining two, the postoperative periods have been only 4 and 6 months.

In the six patients in whom repeated pressure by means of the Frank technique was the principal method of management, satisfactory coitus was possible in the four who have attempted it. In the three patients in whom vaginal depth exceeded 2 cm at the outset and where coitus was practiced as an additional deepening mechanism, doubling of the depth occurred readily and within 2 months. In two patients in whom the vagina originally was less than 1 cm in length, progress was slower and more uncertain, but significant deepening was achieved after 2 to 4 months. In two other patients with uterovaginal agenesis and a vagina of less than 2 cm, progress with dilator pressure proved to be marginal and discouraging, leading to abandonment of the method in favor of the surgical approach.

In the group of eight patients with a functioning uterus in whom vaginal atresia constituted an obstruction to the middle or lower third of the vagina, a pelvic mass became an indication for laparotomy in six. In one instance in a 1-day-old infant, it proved to be a hydrometrocolpos. In the remaining five, the operation was carried out around the time of puberty, and the mass was found to be a hematocolpos. In one, hysterectomy was performed as a primary procedure. In four, the distended vagina was opened from within the peritoneal cavity and a communication was established surgically between the lower pole of the vagina and the



urogenital sinus or distal vagina. This permitted conservation of the functioning uterus while a second, later operation could deal with the atretic vaginal segment. Two of these patients subsequently underwent hysterectomy and partial vaginectomy, but in the other two, the atretic segment was either excised or divided and a satisfactory functioning vagina was established to communicate with a normal upper part of the vagina and uterus. Two girls are still in the pubertal age and a definitive procedure has not been carried out, as it has been elected to await the development of a hematocolpos as a better guide to establishing the limits of the vagina prior to creating a communication for drainage through the atretic segment.

#### Comment

A plan of management of congenital absence of the vagina requires careful assessment of the extent of the müllerian tract abnormality and a determination by clinical examination and/or laparoscopy of whether or not a uterus capable of function is present. Because of the frequent association of multiple developmental defects, a careful search should be made for abnormalities of other body systems. The incidence and the most probable site of the abnormalities vary with the clinical syndrome of the individual patient. In uterovaginal agenesis, the reported predisposition to anomalies of the urinary and skeletal variety<sup>4</sup> was supported by the findings of this study, where abnormalities of the kidneys and collecting system were noted in five of the 19 patients. A single kidney was present in four, and in two instances, it was located in the pelvis. Skeletal anomalies were noted in four of the 19 women. In the 10 patients in whom a functioning uterus was present, a major anomaly of the urinary or anorectal tract coexisted in six.

Management of congenital absence of the vagina can only be planned with a full understanding of the nature and extent of other anatomic, genetic, and metabolic disturbances. Optimally, the nature of the vaginal abnormality should be evaluated as well as possible and discussed with the patient and the parents at an early age so that the psychological preparation of the patient for her limitation can be instituted and a plan for appropriate timed intervention can be developed. Reconstructive surgery of the genital tract should not be performed in the prepubertal child as it can better be deferred close to the time of sexual activity. Premature efforts merely contribute scarring to the difficulties of later surgery.

Since the publications of 1938<sup>6</sup> and 1950,<sup>7</sup> of McIndoe, describing the split-thickness skin inlay grafting technique of a newly created vaginal space, all previous

operative techniques have become of historical interest. This operation has become the standard against which alternative methods of management may be judged. In earlier series,<sup>2, 7, 8</sup> significant morbidity and damage to the bowel and bladder were recorded, but with the use of pliable molds and close supervision of the healing of the vaginal space, morbidity has been markedly reduced. During a contractile period that may last for 4 to 6 months, inlay grafts are prone to contract, after which they cannot be made to expand. It follows that the mold must provide constant, not intermittent, distention of the vaginal space during this phase. A major responsibility falls to the patient to wear the mold conscientiously and carry out the daily removal and reinsertion of the mold following flushing of the vagina. An intelligent and conscientious patient can readily carry out this important part of her postoperative management. The operation is ideally reserved for responsible, motivated young women who are planning marriage in 2 to 4 months. The operation has been performed where there is no such prospect of a regular sexual relationship, but in this circumstance, the wearing of the mold, at least occasionally on an ongoing basis, becomes important to guard against the prospect of late contraction of the vaginal skin. It is clearly wrong to undertake the operation where the patient is too emotionally immature or otherwise incapable of playing her essential role.

In 1964, Williams<sup>11</sup> described an operation that has gained considerable acceptance as a simpler alternative to the McIndoe operation.<sup>2</sup> A vulvovaginal pouch is created and lined by skin of the labia majora and perineum. Although the pouch is not an anatomically true vagina, it does deepen and assume a more normal axis with use. It has the advantage that contracture does not occur and the wearing of the mold or the use of dilators is unnecessary. This represents a substantial advantage and provides greater flexibility in the timing of the procedure. In addition to the low morbidity and short hospital stay of the Williams vulvovaginoplasty, a further advantage is its use in selected patients with abnormal or ambiguous external genitalia, as was the case in one patient in this series. It may also be utilized following an unsuccessful McIndoe operation where scarring and contracture have led to shortening or vaginal occlusion.

The Frank or directed-pressure technique has particular attraction where there is already a vagina of 2 to 4 cm, as is commonly seen in the testicular feminization syndrome. Good results have been reported<sup>10</sup> where the vagina is less than 1 cm in length, but this technique has less applicability where the vagina is short, as is the situation in many women with uterovaginal agenesis.

However, the simplicity of this method makes it useful to consider as the initial approach in most women in whom the uterus is absent.

It is important to acknowledge that no treatment may be needed in many women where the initial vaginal depth measures 3 to 4 cm or greater. Following minor initial coital discomfort, the vagina deepens and symptoms disappear.

The treatment plan for any patient with congenital absence of the vagina must be individualized. Optimal results are achieved where there is due consideration of the psychological and social factors as well as the anatomic problem. All treatment choices are rendered more likely to succeed if there is provided a program of ongoing supervision and support that may extend over many months.

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### **Changes in the milk composition of nonpuerperal women**

Jerzy K. Kulski, Ph.D., Peter E. Hartmann, Ph.D., Warren J. Saint, B.Med.Sci.,  
Patrick F. Giles, M.Sc., M.B., F.R.C.O., and Donald H. Gutteridge, M.B., F.R.A.C.P.  
*Nedlands, Western Australia*



# "MENSTRUAL CRAMPS"

NOW...

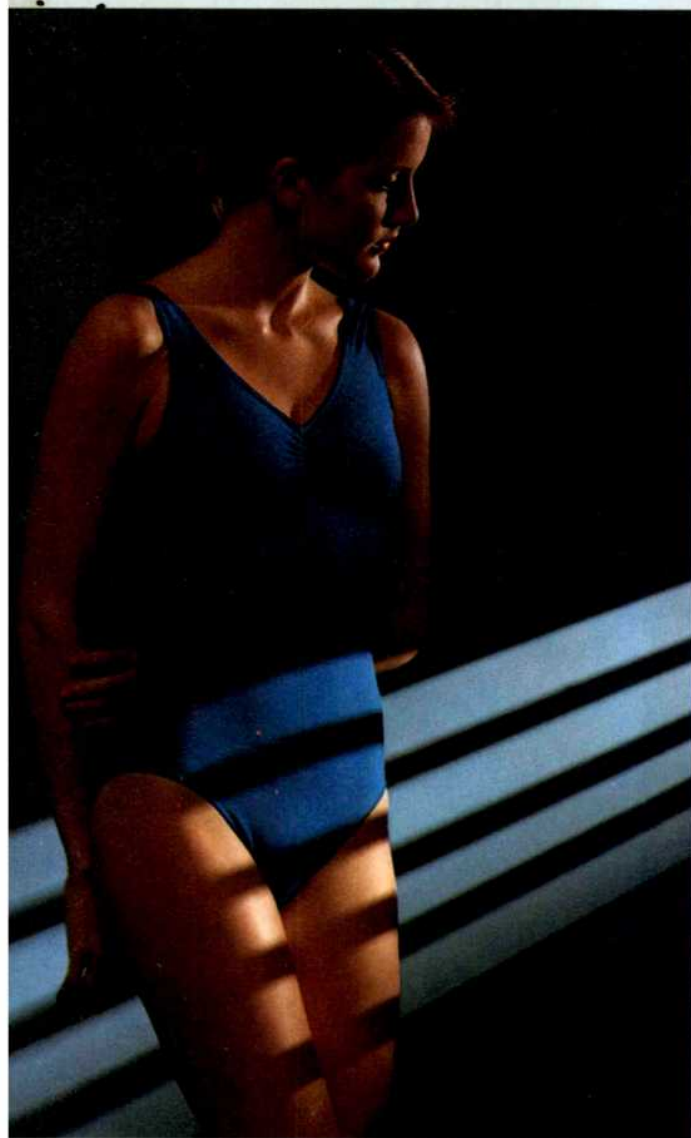


**A NEW  
THERAPEUTIC  
ALTERNATIVE\***

NEW from SYNTEX RESEARCH

Please see full Prescribing Information on last page.

# DYSMEN



275mg TABLETS

## NEW

# ANAPROX<sup>®</sup>

## (NAPROXEN SODIUM)

A new therapeutic alternative



# DYSMENORRHEA

## A NON-NARCOTIC, SINGLE-ENTITY ORAL ANALGESIC\*

New Anaprox® (naproxen sodium) is an effective, mid-range analgesic that has been proven to be useful in the treatment of primary dysmenorrhea. Anaprox represents a new therapeutic alternative for obstetricians/gynecologists who frequently encounter primary dysmenorrhea in practice.

## FULL THERAPEUTIC EFFECT IN ONE HOUR

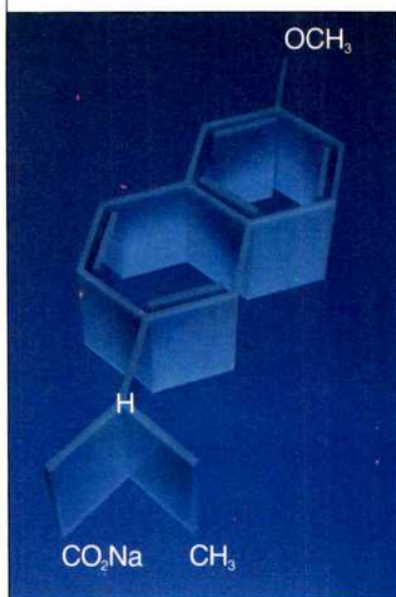
With Anaprox, full therapeutic effect is reached within one hour. Peak plasma levels are attained in 1-2 hours. Anaprox also offers a longer half-life than other non-hormonal agents indicated for dysmenorrhea. This helps sustain relief of painful dysmenorrhea from dose to dose, whether administered t.i.d. or q.i.d.

## PROVIDES FAST, LONG-LASTING RELIEF OF PAIN AND UTERINE CRAMPING

Rapid absorption and fast onset of action help assure

rapid relief of the distressing symptoms of dysmenorrhea—pain and uterine cramping.

Anaprox  
Chemical Structure



## AN EFFECTIVE INHIBITOR OF PROSTAGLANDIN SYNTHETASE AND UTERINE CONTRACTILITY

Anaprox, a member of the arylacetic acid class of drugs, is an effective inhibitor of prostaglandin synthetase. In patients studied, it has been shown that Anaprox significantly reduces the level of prostaglandins in the uterus, which is accompanied by significant reduction in uterine contractions. Reduced con-

tractility results in decreased painful cramping of dysmenorrhea.

## WELL TOLERATED BY MOST PATIENTS

Anaprox was well tolerated in most patients studied. In fact, studies showed a lower incidence of GI and CNS side effects when it was compared with aspirin. With Anaprox, the most common adverse reactions were mild GI and CNS side effects, which occurred in approximately 10% and 11%, respectively. Use with caution in patients with impaired renal function. Because of adverse eye changes with NSAIs, periodic ophthalmic studies should be carried out. Peripheral edema has been observed in some patients. Because of its antipyretic properties, Anaprox may mask the signs and symptoms of infection. Before prescribing, please see warnings, precautions, and adverse reactions sections of full Prescribing Information.

Before initiating Anaprox therapy, it should be deter-

**\*Anaprox is a nonsteroidal anti-inflammatory agent, which has been developed as an analgesic and possesses antipyretic properties.**

Please see full Prescribing Information on last page.



# DYSMEN

mined that the patient is not pregnant.

Anaprox should not be used concomitantly with the related drug Naprosyn® (naproxen), since both circulate in plasma as the naproxen anion and have the same side effects.

## EXTENSIVE CLINICAL DOCUMENTATION

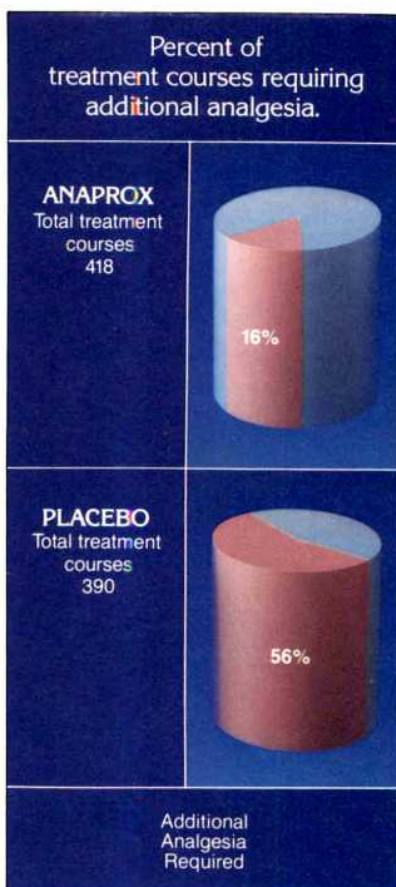
Anaprox has been the subject of extensive clinical investigation in dysmenorrhea, having been studied in 212 patients over 496 menstrual cycles.

## EFFICACY DEMONSTRATED BY RELIEF OF MENSTRUAL PAIN

In eleven double-blind parallel or cross-over studies, and one single-blind study, involving 212 patients over 496 treatment courses, patients in 63% of the treatment courses derived "substantial to complete relief" and 11% experienced "mild to substantial relief" with Anaprox. Researchers concluded that "... a significant and beneficial effect of Anaprox on menstrual pain can be expected in about 75% of the treatment courses."

## EFFICACY DEMONSTRATED BY REDUCED NEED FOR ADDITIONAL ANALGESIA

The eleven double-blind comparison studies with placebo were conducted over 808 treatment courses. In 418 Anaprox treatment courses, only 16% required additional pain medication. In 390 placebo treatment courses, however, additional analgesia was required in 56% of the treatment courses.



## EFFICACY DEMONSTRATED BY LESS DAILY ACTIVITY INTERFERENCE

Continuation of patients' usual daily activities during painful menstruation was evaluated in a double-blind study involving 64 patients given Anaprox or placebo. Scores were totaled over three treatment courses. The study showed conclusively that those patients treated with Anaprox experienced substantially less interference with usual daily activities than those treated with placebo.

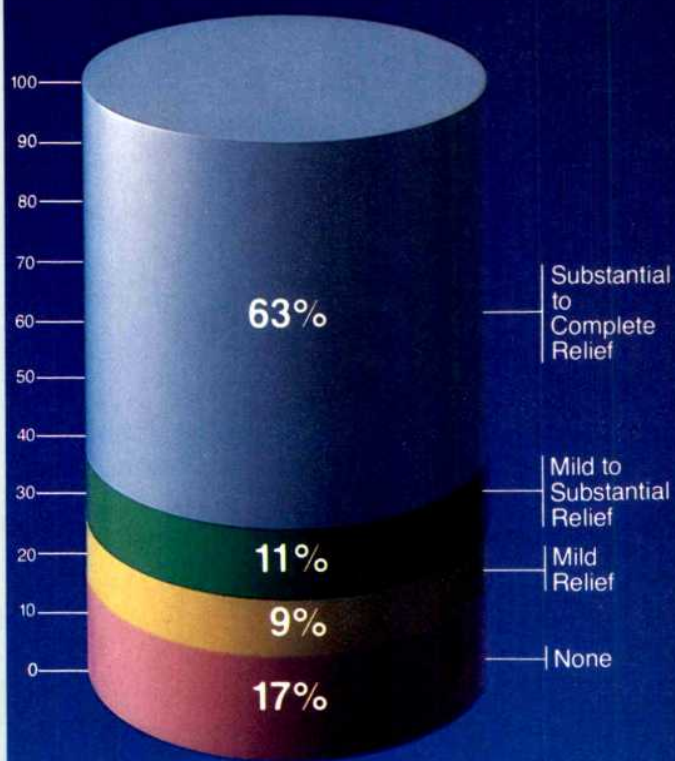
## EFFICACY DEMONSTRATED BY DECREASED INTRAUTERINE PRESSURE

Anaprox efficacy in dysmenorrhea has been further documented by measured decrease in uterine pressure. Measurement was conducted using a microballoon-tipped catheter introduced transcervically into the uterine cavity and connected to a recording system. Patients treated with Anaprox showed a significant decrease in resting pressure, amplitude, and frequency of uterine contractions.



# DORRHEA

Degree of menstrual pain relief  
achieved with Anaprox



## NEW

275mg TABLETS

# ANAPROX<sup>®</sup>

(NAPROXEN SODIUM)

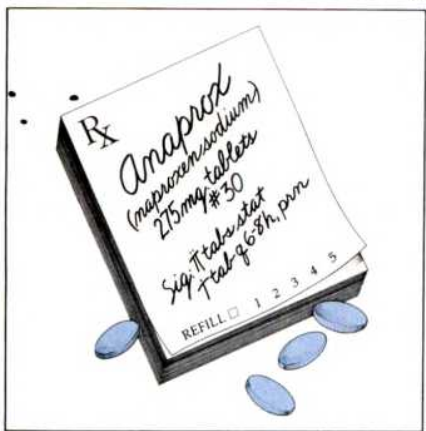
## A new therapeutic alternative

Please see full Prescribing Information on last page.



FOR DYSMENORRHEA

# NEW 275 mg tablets ANAPROX® (NAPROXEN SODIUM)



**Description:** ANAPROX filmcoated tablets for oral administration each contain 275 mg of naproxen sodium, which is equivalent to 250 mg naproxen with 25 mg (about 1 mEq) sodium. It is related to the arylacetic acid class of drugs. ANAPROX is a non-narcotic analgesic agent with anti-inflammatory and antipyretic actions. These properties have been demonstrated in classical animal test systems.

The chemical name of naproxen sodium is (-)-sodium 2-(6-methoxy-2-naphthyl) propionate. Naproxen sodium is a white to creamy white, crystalline solid, freely soluble in water.

**Clinical Pharmacology:** Naproxen sodium is rapidly and completely absorbed from the gastrointestinal tract. After administration of naproxen sodium, peak plasma levels of naproxen anion are attained at 1-2 hours. The mean biological half-life of the anion in humans is approximately 13 hours, and at therapeutic levels it is greater than 99% albumin bound. Approximately 95% of the dose is excreted in the urine, primarily as naproxen, 6-O-desmethyl naproxen or their conjugates. The rate of excretion has been found to coincide closely with the rate of drug disappearance from the plasma. The drug does not induce metabolizing enzymes. Naproxen anion inhibits prostaglandin synthesis but beyond this its mode of action is unknown.

The drug was studied in patients with mild to moderate pain, and pain relief was obtained within 1 hour. It is not a narcotic and is not a CNS-acting drug. Controlled double-blind studies have demonstrated the analgesic properties of the drug in, for example, post-operative, post-partum, orthopedic and uterine contraction pain and dysmenorrhea. In dysmenorrheic patients, the drug reduces the level of prostaglandins in the uterus, leading to a reduction in the frequency and severity of uterine contractions. Analgesic action has been shown by such measures as reduction of pain intensity scores, increase in pain relief scores, decrease in numbers of patients requiring additional analgesic medication, and delay in time for required remediation. The analgesic effect has been found to last for up to 7 hours.

The drug was studied in patients with rheumatoid arthritis and osteoarthritis. It is not a corticosteroid. Improvement in patients treated for rheumatoid arthritis has been demonstrated by a reduction in joint swelling, a reduction in pain, a reduction in duration of morning stiffness, a reduction in disease activity as assessed by both the investigator and patient, and by increased mobility as demonstrated by a reduction in walking time.

In patients with osteoarthritis, the therapeutic action of the drug has been shown by a reduction in joint pain or tenderness, an increase in range of motion in knee joints, increased mobility as demonstrated by a reduction in walking time, and improvement in capacity to perform activities of daily living impaired by the disease.

In clinical studies in patients with rheumatoid arthritis and osteoarthritis, the drug has been shown to be comparable to aspirin in controlling the aforementioned measures of disease activity, but the frequency and severity of the milder gastrointestinal adverse effects (nausea, dyspepsia, heartburn) and nervous system adverse effects (tinnitus, dizziness, light-headedness) were less than in the aspirin-treated patients. It is not known whether the drug causes less peptic ulceration than aspirin.

The drug may be used safely in combination with gold salts and/or corticosteroids; however, in controlled clinical trials, when added to the regimen of patients receiving corticosteroids it did not appear to cause greater improvement over that seen with corticosteroids alone. Whether the drug could be used in conjunction with partially effective doses of corticosteroid for a "steroid-sparing" effect has not been adequately studied. When added to the regimen of patients receiving gold salts, the drug did result in greater improvement. Its use in combination with salicylates is not recommended because data are inadequate to demonstrate that the drug produces greater improvement over that achieved with aspirin alone. Further, there is some evidence that aspirin increases the rate of excretion of the drug.

Generally, improvement due to the drug has not been found to be dependent on age, sex, severity or duration of disease.

In <sup>51</sup>Cr blood loss and gastroscopy studies with normal volunteers, daily administration of 1100 mg of ANAPROX (naproxen sodium) has been demonstrated to cause statistically significantly less gastric bleeding and erosion than 3250 mg of aspirin.

**Indications and Usage:** ANAPROX® (naproxen sodium) is indicated in the relief of mild to moderate pain and for the treatment of primary dysmenorrhea.

It is also indicated for the treatment of the signs and symptoms of rheumatoid arthritis and osteoarthritis.

The safety and effectiveness of the drug in children have not been established.

**Contraindications:** Naproxen sodium is contraindicated in patients who have shown hypersensitivity to it. Because the potential exists for cross-sensitivity reactions, it should not be given to patients in whom aspirin or other non-steroidal, anti-inflammatory drugs induce the syndrome of asthma, rhinitis, or urticaria.

**Warnings:** Gastrointestinal bleeding, sometimes severe, and occasionally fatal, has been reported in patients receiving the drug. Among 960 patients treated for rheumatoid arthritis or osteoarthritis during the course of clinical trials in the United States (260 treated for more than two years), 16 cases of peptic ulceration were reported. More than half were on concomitant corticosteroid and/or salicylate therapy and about a third had a prior history of peptic ulcer. Gastrointestinal bleeding, including nine potentially serious cases, was also reported in this population. These were not always preceded by premonitory gastrointestinal symptoms. Although most of the patients with serious bleeding were receiving concomitant therapy and had a history of peptic ulcer disease, it should be kept in mind that the drug also has the potential for causing gastrointestinal bleeding on its own. Therefore, it should be administered to patients with active gastric and duodenal ulcers only under close supervision.

**Precautions:** General: ANAPROX® (NAPROXEN SODIUM) SHOULD NOT BE USED CONCOMITANTLY WITH THE RELATED DRUG NAPROSYN® (NAPROXEN) SINCE THEY BOTH CIRCULATE IN PLASMA AS THE NAPROXEN ANION.

Because of adverse eye findings in animal studies with drugs of this class, it is recommended that ophthalmic studies be carried out within a reasonable period of time after starting therapy and at periodic intervals thereafter if the drug is to be used for an extended period of time.

In chronic studies in laboratory animals, the drug has caused nephritis. Glomerular nephritis, interstitial nephritis and nephrotic syndrome have been reported. Since the drug is eliminated to a large extent from the body by urinary excretion via glomerular filtration, this drug should be used with great caution in patients with significantly impaired renal function and the monitoring of serum creatinine and/or creatinine clearance is advised in these patients. In clinical trials a few patients developed mild elevations in BUN accompanied by no other signs and symptoms.

If steroid dosage is reduced or eliminated during therapy, the steroid dosage should be reduced slowly and the patients must be observed closely for any evidence of adverse effects, including adrenal insufficiency and exacerbation of symptoms of arthritis.

Patients with initial hemoglobin values of 10 grams or less who are to receive long-term therapy should have hemoglobin values determined frequently.

Peripheral edema has been observed in some patients. Since each naproxen sodium tablet contains approximately 25 mg (about 1 mEq) of sodium, this should be considered in patients whose overall intake of sodium must be markedly restricted. It is possible that patients with questionable or compromised cardiac function may be at a greater risk when taking the drug.

**Information for Patients:** Caution should be exercised by patients whose activities require alertness if they experience drowsiness, dizziness, vertigo or depression during therapy with the drug.

**Drug Interactions:** *In vitro* studies have shown that naproxen anion, because of its affinity for protein, may displace from their binding sites other drugs which are also albumin-bound. Theoretically, the naproxen anion itself could likewise be displaced. Short-term controlled studies failed to show that taking the drug significantly affects prothrombin times when administered to individuals on coumarin-type anticoagulants. Caution is advised nonetheless, since interactions have been seen with other nonsteroidal agents of this class. Similarly, patients receiving the drug and a hydantoin, sulfonamide or sulfonylurea should be observed for signs of toxicity to these drugs.

Probenecid given concurrently increases naproxen anion plasma levels and extends its plasma half-life significantly.

**Drug/Laboratory Test Interactions:** The drug may decrease platelet aggregation and prolong bleeding time. This effect should be kept in mind when bleeding times are determined.

The administration of the drug may result in increased urinary values for 17-ketogenic steroids because of an interaction between the drug and/or its metabolites with m-dinitro-benzene used in this assay. Although 17-hydroxy-corticosteroid measurements (Porter-Silber test) do not appear to be artifactually altered, it is suggested that therapy with the drug be temporarily discontinued 72 hours before adrenal function tests are performed.

The drug may interfere with some urinary assays of 5-hydroxy indoleacetic acid (SHIAA).

**Carcinogenesis:** A two-year study was performed in rats to evaluate the carcinogenic potential of the drug. No evidence of carcinogenicity was found.

**Pregnancy:** Teratogenic Effects: Pregnancy Category B. Reproduction studies have been performed in rats, rabbits

and mice at doses up to six times the human dose and have revealed no evidence of impaired fertility or harm to the fetus due to the drug. There are, however, no adequate and well-controlled studies in pregnant women. Because animal reproduction studies are not always predictive of human response, the drug should not be used during pregnancy unless clearly needed.

**Non-teratogenic Effects:** In rats, pregnancy was prolonged when the drug was given before the onset of labor; where it was given after the delivery process had begun, labor was protracted.

**Nursing Mothers:** Caution should be exercised if the drug is administered to a nursing woman since the naproxen anion has been found in the milk of lactating women at a concentration of approximately 1% of that found in the plasma.

**Pediatric Use:** Pediatric indications and dosage have not been established.

**Adverse Reactions:** Adverse reactions reported in controlled clinical trials in 960 patients treated for rheumatoid arthritis or osteoarthritis are listed below. In general, these reactions were reported 2 to 10 times more frequently than they were in studies in the 962 patients treated for mild to moderate pain or for dysmenorrhea.

**Incidence Greater Than 1%: Gastrointestinal:** The most frequent complaints reported related to the gastrointestinal tract. They were: constipation,\* heartburn,\* abdominal pain,\* nausea,\* dyspepsia, diarrhea, stomatitis.

**Central Nervous System:** Headache,\* dizziness,\* drowsiness,\* lightheadedness, vertigo.

**Dermatologic:** Itching (pruritis),\* skin eruptions,\* ecchymoses,\* sweating, purpura.

**Special Senses:** Tinnitus,\* hearing disturbances, visual disturbances.

**Cardiovascular:** Edema,\* dyspnea,\* palpitations.

**General:** Thirst.

\*Incidence of reported reaction between 3% and 9%. Those reactions occurring in less than 3% of the patients are unmarked.

**Incidence Less than 1%: Probable Causal Relationship:** The following adverse reactions were reported less frequently than 1% during controlled clinical trials and through voluntary reports since marketing. The probability of a causal relationship exists between the drug and these adverse reactions: congestive heart failure, renal disease, glomerular nephritis, interstitial nephritis, nephrotic syndrome, abnormal liver function tests, hematuria, jaundice, thrombocytopenia, leukopenia, granulocytopenia, gastrointestinal bleeding, peptic ulceration with bleeding and/or perforation, hematemesis, melena, vomiting, eosinophilia, pyrexia (chills and fever), skin rashes, menstrual disorders, myalgia and muscle weakness, alopecia, inability to concentrate, depression, malaise, dream abnormalities.

**Causal Relationship Unknown:** Other reactions have been reported in circumstances in which a causal relationship could not be established. However, in these rarely reported events, the possibility cannot be excluded. Therefore these observations are being listed to serve as alerting information to the physicians: angioneurotic edema, agranulocytosis, aplastic anemia, hemolytic anemia, hypoglycemia, hyperglycemia, urticaria.

**Overdosage:** Significant overdosage may be characterized by drowsiness, heartburn, indigestion, nausea or vomiting. Because naproxen sodium may be rapidly absorbed, high and early blood levels should be anticipated. No evidence of toxicity or late sequelae have been reported 5 to 15 months after ingestion for three to seven days of doses equivalent to up to 3,300 mg of naproxen sodium. One patient ingested a single dose equivalent to 27.5 g of naproxen sodium and experienced mild nausea and indigestion. It is not known what dose of the drug would be life threatening. The oral LD<sub>50</sub> of the drug is 543 mg/kg in rats, 1234 mg/kg in mice, 4110 mg/kg in hamsters and greater than 1000 mg/kg in dogs.

Should a patient ingest a large number of tablets, accidentally or purposefully, the stomach may be emptied and usual supportive measures employed. Animal studies suggest that the prompt administration of 5 grams of activated charcoal would tend to reduce markedly the absorption of the drug. It is not known if the drug is dialyzable.

**Dosage and Administration: For Mild to Moderate Pain and Dysmenorrhea:** The recommended starting dose is two 275 mg tablets, followed by one 275 mg tablet every 6 to 8 hours, as required. The total daily dose should not exceed 5 tablets (1,375 mg).

**For Rheumatoid Arthritis and Osteoarthritis:** The recommended starting dose in adults is one 275 mg tablet twice daily (morning and evening) or one 275 mg tablet in the morning and two 275 mg tablets in the evening. During long-term administration, the dose may be adjusted up or down depending on the clinical response of the patient. A lower daily dose may suffice for long-term administration. Daily doses higher than 1100 mg in these indications have not been studied. The morning and evening doses do not have to be equal in size and the administration of the drug more frequently than twice daily is not necessary. Symptomatic improvement in arthritis usually begins within two weeks. However, if improvement is not seen within this period, a trial for an additional two weeks should be considered.

**How Supplied:** ANAPROX® (naproxen sodium) is available in filmcoated tablets of 275 mg (light blue), in bottles of 100 tablets (NDC 18393-274-42) and 500 tablets (NDC 18393-274-62). Store at room temperature in well-closed containers.



Syntex Puerto Rico, Inc.  
Humacao, P.R. 00661



# Endometrial neoplasia—Screening the high-risk patient

JOSEPH H. CRAMER, M.D.C.M., F.R.C.S.(C.)\*

RAYMOND J. OSBORNE, M.D., F.R.C.S.(C.)

*Toronto, Ontario, Canada*

Increased longevity and use of exogenous estrogens appear to have placed more women at risk of developing endometrial carcinoma. Too infrequently, a diagnosis is made in these women at the premalignant stage of their disease. We assessed cytologically, by means of the Milan-Markley helix technique, 170 women seen at our institutions. These patients were classified in one of the following groups: late menstruators, periclimacteric bleeders, women receiving hormone replacement therapy, and women with endometrial pathologic conditions at the time of curettage. The diagnostic accuracy of the helix technique was assessed. Histologic confirmation was obtained with either Vabra or formal curettage. Tolerance on the part of the patient was excellent. Discomfort sufficient to terminate the procedure occurred in two of 189 samples. There were no complications. Tissue adequate for diagnosis was obtained in 180 of 189 samples. Sensitivity was 97%, specificity was 96%, and predictive value of a positive result was 97%. No cases of adenocarcinoma failed to be detected. We conclude that the Milan-Markley helix technique is safe and dependable in assessing women at risk of developing uterine pathologic conditions. (AM. J. OBSTET. GYNECOL. 139:285, 1981.)

ENDOMETRIAL CANCER and its precursors exfoliate cells into the endometrial cavity. Various methods<sup>1-6</sup> of obtaining specimens for cytologic study have been described over the past 40 years. The problem of collecting and studying these cells was alluded to over 20 years ago. Nieburgs<sup>7</sup> wrote, "These difficulties in evaluation of endometrial cells are due to the fact that with most techniques of cell collection, the cells obtained have undergone post-exfoliative changes. When such cells have undergone post-exfoliative deterioration the differential diagnosis by microscopic evaluation may be difficult. Perhaps the development of techniques for the collection of abraded, rather than exfoliative cells, may produce more satisfactory results."

The best method available at present for obtaining abraded cells and tissue is formal curettage. This diagnostic gynecologic procedure is the one most fre-

quently performed, and it accounts for a large proportion of hospital bed usage and operating time. This procedure is not without inconvenience and risk, which is even more substantial when it is done under general anesthesia, and it is inappropriate in the repeated follow-up of high-risk patients.

We have analyzed the usefulness, specificity, sensitivity, and predictive value of a simple outpatient method of endometrial sampling in women at high risk for endometrial abnormalities. The Milan-Markley (Mi-Mark) helix technique allows for the study of directly smeared tissue obtained from within the uterine cavity, thus eliminating filtering, centrifugation, and complex processing. The helix technique acquires living cells, and, at times, tiny surface microanatomic structures, both of which lend themselves effectively to cytologic study. The Mi-Mark helix procedure was performed on 170 women, and a total of 189 specimens was obtained. The results indicate that this method is a reliable alternative to other outpatient methods of endometrial sampling.

## Material and methods

One hundred and seventy women were selected from those attending a special endometrial cytology clinic at the Toronto General Hospital or from inpa-

*From the Ontario Cancer Institute, The Princess Margaret Hospital, and the Toronto General Hospital.*

*Presented at the Thirty-sixth Annual Meeting of the Society of Obstetricians and Gynaecologists of Canada, Jasper, Alberta, Canada, June 10-14, 1980.*

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*\*Gordon Richards Fellow.*

**Table I.** Distribution of patients by age

| Age (yr) | No. |
|----------|-----|
| 20-29    | 4   |
| 30-39    | 5   |
| 40-49    | 28  |
| 50-59    | 48  |
| 60-69    | 51  |
| >70      | 34  |
| Total    | 170 |

**Table II.** Distribution by risk group

| Risk group                                     | No. |
|------------------------------------------------|-----|
| Postmenopausal bleeders                        | 53  |
| Late menstruators                              | 18  |
| Women on hormone replacement therapy           | 41  |
| Dysfunctional bleeders                         | 33  |
| Patients with endometrial pathologic condition | 25  |
| Total                                          | 170 |

tients at The Princess Margaret Hospital, Toronto, Canada. They were sampled a total of 189 times by means of the Mi-Mark technique. Their stratification by age is shown in Table I. The women sampled were among those who are generally considered to be at high risk for endometrial abnormalities.<sup>11</sup> In general, they fall into one of the following broad clinical groupings: (1) postmenopausal bleeders, (2) women on hormone replacement therapy, (3) late menstruators, (4) dysfunctional bleeders, and (5) patients with previously diagnosed endometrial pathology (Table II). Endometrial abnormalities, as we defined them, included endometrial hyperplasia, adenomatous endometrial hyperplasia, with or without atypia, and carcinoma, either in situ or invasive.

A complete history was taken, with particular attention to previous gynecologic operations, and to the possibility of intrauterine pregnancy. A careful pelvic examination was carried out. Sampling of material for the cervicovaginal smear was acquired in the usual manner. The cervix was then cleansed of residual cells and mucus.

The sampling technique used was the same as that described by Milan and Markley.<sup>1, 2, 8</sup> Briefly, after the cervix had been cleansed, and it had been immobilized with a single-toothed tenaculum if necessary, the spiral was introduced to the uterine fundus. Rarely was dilatation of the os necessary, but if required, it was done by means of the 3.5 mm plastic dilator handle of the "paddle." The helix was twisted 10 times clockwise and was withdrawn as the twisting motion continued. The extruder-paddle was then used to remove the specimen and place it on a slide which was thoroughly wetted

with 95% ethyl alcohol. Once on the slide, the specimen was spread evenly, placed in a jar of Carnoy's solution for 30 minutes, fixed, and stained.

The cytologic specimens obtained were all processed and examined by a single pathologist at the Toronto General Hospital. Standard criteria for cytologic atypia were used in classifying these samples. Abnormal processes were indicated by some of the following features: enlarged nuclei, multiple nucleoli, chromatin clumping, irregular nuclear borders, irregular thickening or budding nuclear membranes, nuclear crowding, altered nuclear-cytoplasmic ratios, and cytoplasmic vacuoles. The degree of cellular abnormality was classified into benign hyperplasia, adenomatous hyperplasia, and atypical adenomatous hyperplasia, with the latter two being graded into mild, moderate, and severe abnormalities.

Of these 170 women, 148 were sampled concurrently or within a month's time of helix sampling by either Vabra suction curettage or formal fractional curettage. Hysterectomy after curettage was carried out in nine patients, and pathologic study was done at the same institution. Direct comparison between the results of helix sampling and these other methods was then made.

## Results

One hundred and seventy women were sampled 189 times by means of the helix. Pathologic confirmation by means of one of the other described methods was made in 148 instances.

**Patient compliance.** Severe pain necessitated termination of two attempts at sampling. Local anesthetic was needed in eight of the 189 sampling procedures. Dilators were needed in seven of 189 procedures. No uterine perforations were recognized to have occurred. No patient experienced a syncopal episode, but 14 patients experienced nausea. No patient vomited during or after the procedure. Slight bleeding from the uterus occurred in 49 out of 189 samplings.

Tissue adequate for diagnosis was obtained in 182 of 189 samplings. Of the 148 patients in whom a pathologic diagnosis was obtained, the following cytologic diagnoses were made. Seventy-six helix samples were interpreted as showing no abnormal cells, 37 had cells or cellular structures consistent with endometrial hyperplasia, 11 were consistent with mild atypical adenomatous endometrial hyperplasia, five with moderate, and five with severe. Two samples were interpreted as showing benign squamous metaplasia, and nine cases were interpreted as adenocarcinoma of the endometrium.

The diagnoses made on samples obtained by curet-



Table III. Predictive value of the helix samples applied to healthy and diseased populations

|                     | Helix                    |                          | Totals                  |
|---------------------|--------------------------|--------------------------|-------------------------|
|                     | No. with positive result | No. with negative result |                         |
| No. with disease    | TP (69)                  | FN (2)                   | TP + FN (71)            |
| No. without disease | FP (3)                   | TN (74)                  | TN + FP (77)            |
| Totals              | FP + TP (72)             | TN + FN (76)             | TP + FP + TN + FN (148) |

Predictive value of a positive result:  $\frac{TP}{TP + FP} \times 100$  or  $\frac{69}{72} \times 100 = 96\%$ . Predictive value of a negative result:  $\frac{TN}{FN + TN} \times 100$  or  $\frac{74}{76} \times 100 = 97.3\%$ .

TP = true positive; FP = false positive; TN = true negative; FN = false negative.

tage are as follows: 77 patients had normal endometrium, 38 had endometrial hyperplasia, 13 had mild, five had moderate, and six had severe atypical adenomatous hyperplasia; nine cases were reported to be adenocarcinoma of the endometrium. Two cases of abnormal endometrium failed to be detected by the helix: one was interpreted as mild atypical adenomatous hyperplasia, and the other as severe atypical adenomatous hyperplasia. In these cases; the helix cytologic findings were reported as, "no abnormal cells present." Three cases were found to be abnormal by the helix sampling method. In all three, the diagnosis was "groups of cells consistent with focal, mild endometrial hyperplasia." Curettage specimens were reported to be normal in these three cases. All nine cases of adenocarcinoma diagnosed pathologically were correctly diagnosed by the helix cytologic specimen.

Comment

The Mi-Mark helix technique was developed in 1973, for sampling the endometrial cavities of women on an outpatient basis, with little discomfort, low cost, and minimal risk. As our results indicate, these practical considerations have been met. Only two attempts at sampling could not be carried out. No serious sequelae or side effects occurred. Compliance and tolerance on the part of the patients were excellent.

Tissue adequate for diagnosis was obtained in 182 of 189 samplings, or 96.3%. This represents results far superior to those recently quoted in the literature,<sup>9</sup> and certainly has much to do with the experience that our cytologist has had with this technique, and his careful and thoughtful interpretation of the samples. In comparison to cytologic findings obtained by Papanicolaou smears in the diagnosis of endometrial lesions, this technique is far superior.

We evaluated this procedure in women who were classically considered to be at high risk for developing endometrial abnormalities, including adenocarcinoma of the endometrium, and have shown that it is a simple

Table IV. False positive, false negative, and predictive values with varying prevalence

| Prevalence | False positives (%) | False negatives (%) | Predictive value of a positive result (%) | Predictive value of a negative result (%) |
|------------|---------------------|---------------------|-------------------------------------------|-------------------------------------------|
| 1/100      | 80.2                | 0.028               | 25                                        | 100                                       |
| 1/50       | 66.8                | 0.058               | 40                                        | 100                                       |
| 1/20       | 43.8                | 0.149               | 63                                        | 100                                       |
| 1/10       | 27.0                | 0.316               | 80                                        | 100                                       |
| 1/5        | 14.1                | 0.708               | 90                                        | 99                                        |
| 1/2        | 3.9                 | 2.774               | 97                                        | 98                                        |

screening test, as it should be. However, a screening test must also effectively divide a population into two groups,<sup>10</sup> one comprised mostly, if not entirely, of individuals who are likely to have disease, and the other comprised mostly, if not entirely, of individuals who are likely to be free of disease.

We examined the predictive value of this test in terms of two questions. How accurately does the test result predict the presence or absence of disease? If there is an abnormal result, what are the chances that it indicates disease? The predictive value of a test is determined by the complex interaction of three variables: the incidence of false negative results in patients with disease, the incidence of false positive results in subjects without disease, and the prevalence of the disease itself in the group examined.

Sensitivity can be defined as the incidence of true positive results in patients with disease. The sensitivity of the Mi-Mark technique was 69/71, or 97%. Specificity can be defined as the incidence of true negative results in subjects without disease. In our study, specificity was 74/77, or 96%. Galen and Gambus<sup>11</sup> defined the predictive value as the percentage of positive results that are true positive when the test is applied to a population comprised of both healthy and diseased individuals. We established that the Mi-Mark helix had a predictive value of 97% (Table III).

There were two instances of false negative results, but a review of these two cases yielded no explanation. Possibly, the area of endometrial surface affected was so small that it was missed by the helix. Moreover, false negative results can occur if the cavity is larger than 4 inches, as was the case in one of the two instances of false negatives in our study.

The results of the present study are not in total agreement with those of a recent study<sup>9</sup> which concluded that, "It is doubtful whether this technique is suitable for widespread screening and monitoring hormone therapy. . . ." The sample size in the present study was small, and this, of itself, precludes recommending this procedure as a widespread screening method; but the results are encouraging. On the basis of our test results and varying prevalence rates, the false negative and false positive incidences shown in

Table IV can be predicted. We can rely upon a negative result, which means that the patient can be reassured and not subjected to further study. Is the Mi-Mark technique like a Papanicolaou test? That is, does the predictive value of a positive result improve with two or more samplings? We are presently carrying out further studies specifically in this area to answer this question. More experience with larger numbers of women may show that this is, indeed, an ideal screening test for the group of women at high risk for endometrial hyperplasia and carcinoma. However, we cannot presently advocate this method as a general screening procedure for women at low risk.

We wish to thank Dr. R. S. Bush, Director, Ontario Cancer Institute, for his assistance in the preparation of this article.

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# Postradiation ureteral obstruction: A reappraisal

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Thirty-four patients who received radiation therapy for carcinoma of the cervix developed hydronephrosis. Twelve of 34 (35%) had no evidence of pelvic malignancy, and the obstruction was caused by periureteral fibrosis. The incidence of obstructive uropathy due to periureteral fibrosis not associated with recurrent tumor increased when the obstructing lesion was unilateral, the clinical staging of the cervical carcinoma prior to therapy was Stage IB or Stage II rather than Stage III or Stage IV, and the obstructing lesion appeared 2 or more years after the completion of radiation therapy. The appearance of hydronephrosis in association with ipsilateral leg edema, and sciatic pain in these patients strongly suggest a recurrent tumor. The diagnosis of periureteral fibrosis should be considered in all patients who develop obstruction of the urinary tract after radiation therapy for carcinoma of the cervix. Laparotomy is indicated if all other methods of investigation fail to confirm the presence of a malignant lesion. (AM. J. OBSTET. GYNECOL. 139:289, 1981.)

IN MOST INSTITUTIONS, radiation remains the primary method of therapy for invasive carcinoma of the cervix. With the improvement in dosimetry and techniques of application, the immediate morbidity associated with therapy decreased.<sup>1</sup> Even so, the rate of complication after radiotherapy is approximately 15%, and it is even higher if supravoltage equipment is used.<sup>1</sup>

The ureters are exposed to radiation damage because of their proximity to the uterine cervix. In most cases, the damage to the tissue bed of the ureter is mild and transient,<sup>2</sup> but in some cases it results in periureteral fibrosis. This fibrous tissue encases and blocks the ureter.<sup>3</sup>

The ureter is apparently more susceptible to radiation damage when there is pelvic cellulitis associated with infiltration and edema of the periureteral tissues, or periureteral involvement by tumor cells.<sup>3, 4</sup>

Permanent damage to the ureters in the form of postradiation fibrosis that leads to obstruction of the ureters has been considered to be rare, with most authors concluding that the incidence of obstructive uropathy caused by fibrosis is approximately 1% (range, 0.8% to 1.6%).<sup>3-8</sup> Therefore, further management has been based on the assumption that obstructive uropathy that occurs after radiation therapy is almost always caused by recurrent carcinoma. However, since 1960, over 100 cases have been reported in which obstructive uropathy occurred as a result of radiation fibrosis without evidence of residual or recurrent malignancy.<sup>3</sup> In two studies,<sup>9, 10</sup> autopsies were performed on patients with carcinoma of the cervix who were given radiation therapy and died of uremia. In all of these patients, the clinical diagnosis was recurrent neoplasm. However, in 12 of 36 patients (33%) in one series and in 18 of 68 patients (26%) in the other, no residual tumor was found at autopsy, and the ureteral obstruction was caused by radiation fibrosis alone.

We have reviewed our experience with ureteral obstruction associated with radiation-treated invasive carcinoma of the cervix, in order to assess the incidence of postradiation fibrosis as the cause of obstructive uropathy, and to identify any clinical features of predictive value in the assessment of the pathogenesis of the obstructive lesion.

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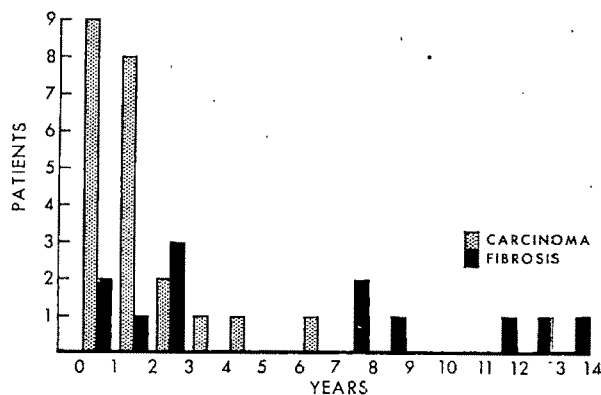


Fig. 1. Cause of ureteral obstruction after therapy.

Table I. Stage of disease at the time of diagnosis related to the cause of ureteral obstruction

|                                                                   | Stage of disease |         |         |         |
|-------------------------------------------------------------------|------------------|---------|---------|---------|
|                                                                   | IB               | II      | III     | IV      |
| No. of patients in whom neoplasm was cause of the obstruction (%) | 2 (23)           | 11 (69) | 4 (100) | 5 (100) |
| No. of patients in whom fibrosis was cause of obstruction (%)     | 7 (77)           | 5 (31)  | 0 (0)   | 0 (0)   |
| Total No. of patients                                             | 9                | 16      | 4       | 5       |

### Material and methods

A review was made of the medical records of all 421 patients who were given radiation therapy for carcinoma of the cervix and were followed at the Ottawa Civic Hospital during the 15-year period 1963 to 1978.

Each patient had a pretreatment evaluation of the urinary tract, including cystoscopy and retrograde pyelography. Twenty-one of the 34 patients who subsequently developed obstructive uropathy also underwent pretreatment intravenous urography. The urinary tract was re-evaluated by intravenous urography at least once during the first 2 years after completion of therapy, and afterward when suggested by symptoms or findings on physical examination.

Most patients were treated by intracavitary radiation (Manchester technique), supplemented by external radiation, cobalt-60 beam, aimed to deliver 7,500 to 8,000 R at Point A and 5,000 to 5,500 at Point B.

In cases of suspected residual disease or an abnormal intravenous urogram, additional investigation, consisting of examination with the patient under anesthesia, cystoscopy, and retrograde pyelography, was carried out. Patients who demonstrated only transient or mild dilatation of the urinary collecting system were not included in the 34 cases studied in greater detail, as outlined hereafter.

During the 15-year period 1963 to 1978, 34 cases of clinically significant hydronephrosis were diagnosed and treated at the Ottawa Civic Hospital. In all of these patients, tissue was obtained for histologic assessment of the nature of the obstructing lesion. Seventeen of 34 patients died and the obstructing lesion was examined at autopsy, at which time the pelvic contents were studied carefully and sections from multiple blocks were interpreted histologically. In 16 patients, assessment of the obstructive lesions was made at laparotomy, and multiple biopsy specimens were obtained. In one patient, cystoscopic examination and biopsy were sufficient to diagnose recurrent cervical carcinoma.

### Results

In four patients, the initial pretreatment evaluation showed signs of obstruction of the urinary tract. All four died of the disease within 6 months, and autopsy revealed that the obstructed ureters were infiltrated by tumor mass.

The other 30 patients, who initially had normal urinary tracts, developed ureteral obstruction at varying intervals after therapy. The shortest period was 3 months, and the longest was 14 years, with a median length of time of 18 months. In nine patients, hydronephrosis developed 4 years or more after the completion of radiation therapy (Fig. 1). Recurrent or residual neoplasm was the cause of ureteral obstruction in 18 of these 30 patients.

In the other 12 patients, the obstruction was the result of periureteral fibrosis. Three of the 12 patients who had ureteral obstruction due to periureteral fibrosis died of neoplastic disease. One patient had extrapelvic metastases from the original cervical carcinoma, with the pelvis free of neoplastic disease. Two patients developed a second primary malignancy. One of these patients developed endometrial adenocarcinoma 14 years after therapy, and an adenocarcinoma of the gastrointestinal tract was found 16 years after therapy in the other. Five patients are alive and well today, 5 years or more after laparotomy.

**Stage of cervical carcinoma.** At the time of diagnosis, the stage of the cervical cancer for the patients in the series was Stage IB for nine patients, Stage II for 16 patients, Stage III for four patients, and Stage IV for the other five patients.

Residual or recurrent neoplasm was the cause of ureteral obstruction in all patients classified as having Stage III and Stage IV disease. In five patients of 16 (31%) with Stage II, and seven patients of nine (77%) with Stage IB disease, the obstruction of the renal tract was the result of periureteral fibrosis (Table I). Appar-



ently, the likelihood of neoplasm obstructing the urinary tract increases when the disease is more advanced at the time of initial therapy and the parametrium is involved.

**Unilateral or bilateral ureteral involvement.** Fourteen of the patients had unilateral ureteral involvement, and 20 patients had both ureters involved. In only three of the 20 patients who had bilateral obstruction was the condition due to periureteral fibrosis. By comparison, in nine patients of 14 with unilateral ureteral obstruction, the condition was due to periureteral fibrosis. Apparently, unilateral involvement is more suggestive of a benign process.

**Time interval between therapy and ureteral obstruction.** In 11 patients, the obstruction became evident during the first year. This group included the four patients who had abnormal urograms prior to therapy and died within 6 months with ureteral obstruction due to tumor. In nine other patients, the obstruction was diagnosed during the second year, and in the remainder (14 patients) the diagnosis was made from 2 years up to 14 years after radiation therapy (Fig. 1). The incidence of periureteral fibrosis rose as the interval between therapy and the appearance of obstruction increased. In only three of 20 patients who had evidence of obstruction of the urinary tract in the first 2 years after therapy was the condition due to periureteral fibrosis. By comparison, in nine of the 14 patients in whom the ureteral obstruction became evident 2 years or more after therapy, the condition was due to periureteral fibrosis. It appears that ureteral obstruction which develops 2 years or more after therapy is more likely to be due to periureteral fibrosis, and less likely to be due to tumor. Furthermore, recurrence of the disease was diagnosed within the first 5 years in 21 of 22 patients (96%).

**Leg edema and sciatic pain associated with hydronephrosis.** Five patients who developed hydronephrosis during the first 2 years after therapy presented with leg edema and sciatic pain. The affected leg was on the same side as the hydronephrosis. All of these patients died within 1 year after the presentation of these complications, and autopsy disclosed that all of them had recurrent or persistent cervical carcinoma. It appears that the triad of hydronephrosis, leg edema, and sciatic pain is indicative of recurrent or persistent malignancy, and is associated with poor prognosis.

**Management of periureteral obstruction.** The management of the 12 patients with periureteral fibrosis was not consistent. Three patients received no therapy. In one of these the clinical diagnosis was recurrent carcinoma; this patient died of uremia, and no malig-

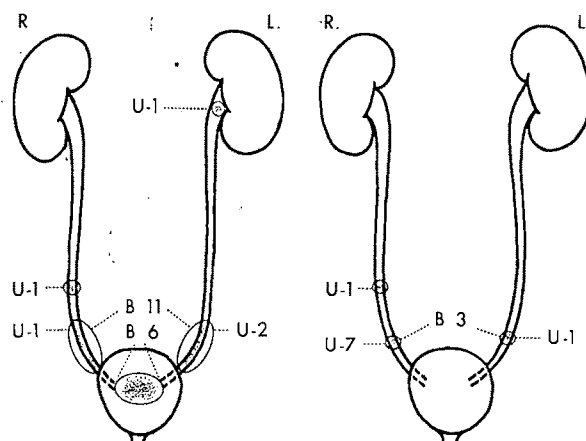


**Fig. 2.** Location of ureteral obstruction. *Left, Cancer. Right, Fibrosis.* U, Unilateral obstruction. B, Bilateral obstruction.

nancy was found at autopsy. The other two patients died before therapy was instituted. In four patients, ureteronephrectomy was performed because of advanced hydronephrotic renal damage. Three patients had ureteral reimplantation to the bladder, and two patients had urinary diversion.

#### Comment

Ureteral obstruction is a recognized complication of extension of cervical carcinoma. In the past, once a patient had ureteral obstruction, she was assumed to have recurrent or persistent carcinoma. In the literature, the incidence of postradiation fibrosis leading to ureteral obstruction ranges between 0.8% and 1.6%.<sup>3-8</sup> This low incidence derives from the fact that most series express the incidence of ureteral obstruction due to postradiation fibrosis as a fraction of the total number of patients treated, rather than relating the number of patients with ureteral obstruction due to postradiation fibrosis only to those patients who developed ureteral obstruction. If only patients with ureteral obstruction are considered, the incidence of ureteral obstruction secondary to postradiation fibrosis rises sharply. In our series, 35% of the patients with obstruction of the urinary tract had periureteral fibrosis. This compares with similar incidence figures of 33% and 26% quoted by the pathologists on autopsy series.<sup>9, 10</sup> In our center, we followed 34 patients with persistent ureteral obstruction after radiation therapy, and histologic assessment of the nature of the obstructive lesion was made in all.

We were able to review the radiation therapy charts of 26 patients. A statistical analysis of the amount of radiation given at Point A and Point B was conducted

by means of Student's *t* test. The mean amount of radiation received by those patients who had periureteral fibrosis was the same as that received by those who did not, at a significance level of  $P = 0.05$ .

The amount of radiation given at Point A did not exceed 8,300 R in all but one patient. This patient received 8,900 R at Point A and developed periureteral fibrosis.

Since all of these patients but one received similar amounts of radiation, within the acceptable therapeutic range, it appears that local factors were probably responsible for the different individual responses to therapy. Two of these local factors, pelvic inflammatory disease and tumor infiltration, are well documented.<sup>3, 4</sup> We can speculate that the increase in the occurrence of pelvic inflammatory disease in the general population may lead in the future to more cases of postradiation fibrosis.

The introduction of newer techniques of raditherapy that utilize high-energy radiation increases the cure rates, but also increases the complication rate, including ureteral obstruction due to postradiation fibrosis.<sup>1, 11</sup>

The availability of cytologic screening for detection of early cervical lesions increased the proportion of patients treated when the disease was localized, namely, Stage I and Stage II. In these groups, the cure rates were 91% and 83%, respectively,<sup>12</sup> and in view of the low recurrence rate the likelihood that the ureteral obstruction is secondary to postradiation fibrosis is high.

We were able to identify clinically significant prognostic signs in identifying patients with ureteral obstruction due to postradiation fibrosis as compared with those in whom the obstruction was due to tumor. The clinical distinction is important with regard to management and prognosis. Stage IB disease was associated with the highest incidence of postradiation fibrosis. Patients with early disease have high cure rates and a low recurrence rate. In these patients, the possibility of ureteral obstruction secondary to recurrent tumor is low.

The time interval between completion of therapy and the appearance of obstructive uropathy is of clinical

importance. A time interval of 2 years or longer is suggestive of periureteral fibrosis. A short time interval may be a reflection of incomplete eradication of the primary tumor. In 96% of our patients with recurrent disease, the malignancy was diagnosed within the first 5 years after therapy. After 5 years, the ureter is unlikely to be obstructed by recurrent disease, and periureteral fibrosis is almost always the cause. In seven patients in our series, ureteral obstruction was diagnosed 5 years or more after completion of therapy. Six of these patients had periureteral fibrosis (86%). In patients with periureteral fibrosis, the location of ureteral obstruction was within 5 cm from the ureteral orifice to the bladder in all but one patient (Fig. 2).

In 14 of 22 patients with recurrent malignancy, the ureter was obstructed in the same location. In six of the other eight patients, the obstruction was at the level of the ureteral orifice (Fig. 2).

A unilateral lesion is more likely to be due to periureteral fibrosis.

The appearance of hydronephrosis associated with ipsilateral leg edema and sciatic pain is strongly suggestive of a recurrent malignant lesion that involves the iliac vessels, lymphatics, and the sciatic nerve. The prognosis in these cases is poor.

In view of the high incidence of postradiation fibrosis as a cause of ureteral obstruction, we think that precise diagnosis of the nature of the obstructing lesion is mandatory.

Clinical assessment in conjunction with cytology, surgical biopsy, needle biopsy, or aspiration biopsy from areas of suspect tumor, cystoscopy, and lymphangiography may all assist in the establishment of a diagnosis of recurrent tumor. However, if such assessment does not establish the diagnosis of recurrent tumor, laparotomy and direct biopsy assessment of the obstructing lesion should be undertaken. Such laparotomy should be performed in an environment in which ureteral obstruction by a benign process can be dealt with surgically in the most appropriate fashion at that time.

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# Risk factors associated with infection following cesarean section

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A retrospective study by means of multivariate discriminant analysis was performed on 496 deliveries (250 vaginal and 246 cesarean sections) to identify risk factors which predispose to postoperative febrile morbidity, in particular, endometritis. The infection rates for endometritis by type of delivery were: vaginal, 3.6%; elective repeat cesarean section, 6.0%; nonurgent primary cesarean section, 22.2%; and emergency cesarean section, 38.4%. No patient-related risk factors were identified for elective repeat cesarean section, and bacterial isolates were most frequently *Staphylococcus aureus*. However, four statistically significant risk factors were associated with the occurrence of endometritis after primary cesarean section. In increasing order of significance, they were duration of labor, number of preoperative vaginal examinations, time membranes were ruptured prior to delivery, and postoperative anemia. Internal fetal monitoring was not a risk factor. The clinical relevance of these findings to the use of prophylactic antibiotics and other attempts aimed at decreasing postoperative morbidity is discussed. (AM. J. OBSTET. GYNECOL. 139:294, 1981.)

AWARENESS of the problem of postoperative infection associated with cesarean section has increased. Recently, investigators have reported rates of febrile morbidity after cesarean section that ranged from 13% to 59%.<sup>1-3</sup> A review of the literature shows that many factors are considered to predispose to such a high rate of infection. These include poor sterile techniques, race,<sup>4</sup> socioeconomic status,<sup>4</sup> maternal age,<sup>5</sup> obesity,<sup>6</sup> lack of prenatal care,<sup>6</sup> prior labor or rupture of membranes,<sup>6-9</sup> number of pelvic examinations preoperatively,<sup>8</sup> and internal fetal monitoring.<sup>9-12</sup>

However, few studies have attempted to differentiate the significance of these various factors. Such differentiation becomes important in view of recent, well-controlled, double-blind studies which advocate that prophylactic antibiotics can reduce the incidence of febrile morbidity after cesarean section. Some authors recommend prophylactic antibiotics for all patients,<sup>13-15</sup>

whereas others restrict their use to selected high-risk patients.<sup>3, 16, 17</sup> Still other authors advocate that strict aseptic techniques can dramatically decrease the rates of postoperative infection without prophylactic antibiotics.<sup>18</sup>

The present study was undertaken to identify more accurately those patients at risk for developing infectious complications after cesarean section, and, furthermore, to determine whether risk factors could be identified in patients which might indicate a benefit from antibiotic prophylaxis at cesarean section.

## Material and methods

The hospital charts of 246 patients who underwent cesarean section from January to August, 1979, were reviewed retrospectively to assess 20 epidemiologic and operative risk factors. An additional 250 patients, selected at random, who underwent vaginal delivery, made up a control group. Relevant data were encoded and keypunched for subsequent computer analysis on an IBM 3033 computer employing subroutines from the Statistical Package for the Social Sciences (SPSS).

Nineteen patients who received antibiotics prior to or within 24 hours after operation were excluded from the study. All medical management, including microbial cultures and antibiotic therapy, was instituted by attending obstetricians. Uterine aspirates were taken

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Table I. Infection rate by type of delivery

|                         | Vaginal<br>(250) | Elective<br>repeat cesarean section<br>(101) | Primary cesarean section |                   |
|-------------------------|------------------|----------------------------------------------|--------------------------|-------------------|
|                         |                  |                                              | Nonurgent<br>(108)       | Emergency<br>(13) |
| Endometritis            | 9 (3.6*)         | 6 (6.0)                                      | 24 (22.2)                | 5 (38.4)          |
| Urinary tract infection | 5 (2.0)          | 11 (10.9)                                    | 5 (4.6)                  | —                 |
| Wound infection         | 10 (4.0)         | 3 (3.0)                                      | 4 (3.7)                  | 2 (15.4)          |

\*Numbers in parentheses are percentages.

Table II. Risk factors associated with endometritis after primary cesarean section

|                              | Incidence of infection |
|------------------------------|------------------------|
| Monitoring                   |                        |
| External                     | 14/61 (23.0)*          |
| Internal                     | 15/59 (25.4)           |
| Duration of labor:           |                        |
| <2 hr                        | 3/22 (13.6)            |
| 2-12 hr                      | 10/44 (22.7)           |
| >12 hr                       | 16/52 (30.8)           |
| No. of vaginal examinations: |                        |
| 0-4                          | 16/79 (21.9)           |
| ≥5                           | 13/42 (30.5)           |
| Time membranes ruptured:     |                        |
| <4 hr                        | 9/51 (17.6)            |
| 4-8 hr                       | 6/23 (26.1)            |
| >8 hr                        | 14/47 (29.7)           |
| Postoperative hemoglobin:    |                        |
| >10.5 mm Hg                  | 17/101 (16.8)          |
| ≤10.5 mm Hg                  | 12/20 (60.0)           |

\*Numbers in parentheses are percentages.

through the cervix and processed for both aerobes and anaerobes by the hospital microbiology department. Routine hematologic investigations were performed prior to delivery, and on the first and third postpartum days.

All patients eligible for the study had at least several prenatal visits with their attending physicians, and most (over 90%) were private, middle-class patients. Of those who required anesthesia, most (over 95%) received epidural anesthesia. For these reasons, prenatal care, socioeconomic status, and anesthesia were not studied as possible risk factors in our series. Where indicated, internal monitoring devices were scalp electrodes and tocodynamometers (i.e., no internal pressure catheters were used).

Febrile morbidity was defined as a temperature in excess of 38° C on at least two successive readings 8 hours apart, excluding the first 24 postoperative hours. Patients who satisfied the above criteria, were subclassified as follows: (1) endometritis—fever, abnormal lochia, or uterine tenderness, with or without positive endometrial cultures; (2) urinary tract infection (UTI)—positive urine culture (>10<sup>5</sup> organisms/ml),

Table III. Microorganisms recovered from infected patients

|                              | Primary<br>cesarean section<br>(29) | Repeat<br>cesarean section<br>(5) |
|------------------------------|-------------------------------------|-----------------------------------|
| Aerobes:                     |                                     |                                   |
| Gram-positive                |                                     |                                   |
| <i>Staphylococcus aureus</i> | 5 (17.2)*                           | 4 (80)                            |
| Streptococci: β-he-          | 5 (17.2)                            |                                   |
| molytic                      |                                     |                                   |
| Other                        | 3 (10.3)                            |                                   |
| Gram-negative                |                                     |                                   |
| <i>Escherichia coli</i>      | 6 (20.7)                            |                                   |
| <i>Proteus mirabilis</i>     | 1 (3.2)                             |                                   |
| Anaerobes:                   |                                     |                                   |
| Gram-positive                |                                     |                                   |
| Peptococcus                  | 1 (3.2)                             |                                   |
| Clostridium                  | 1 (3.2)                             |                                   |
| Gram-negative                |                                     |                                   |
| <i>Bacteroides fragilis</i>  | 11 (37.9)                           | 1 (20)                            |
| Bacteroides—other            | 2 (6.4)                             |                                   |

\*Numbers in parentheses are percentages.

with or without dysuria, with or without fever. (3) wound infection—cellulitis or exudate with positive cultures from incision site, with or without fever. All patients who were considered to have an infection could be grouped according to the above-mentioned classification, and all those with endometritis or urinary tract infections were treated with appropriate antibiotics.

Results

Of 477 patients included in the study, 85 (17.8%) developed postpartum infectious complications. There was a marked difference in the rates of infection for different types of delivery, as outlined in Table I. There was no statistically significant difference in the incidence of infection, in particular, endometritis, for vaginal deliveries (3.6%) and elective repeat cesarean section (6.0%). Both of these rates are comparable to those reported previously from various centers.<sup>2, 17, 18, 20</sup> However, the incidence of infection, in particular, endometritis, after primary cesarean section (23.9%) was significantly increased when compared to the rates for the former two groups (p < 0.01). Noteworthy is the higher

Table IV

|                                 | Hawrylyshyn <i>et al.</i>     | Gibbs <i>et al.</i> <sup>11,20</sup> | Green <i>et al.</i> <sup>12,16</sup> |
|---------------------------------|-------------------------------|--------------------------------------|--------------------------------------|
| Study population                | 126 primary cesarean sections | 419 cesarean sections                | 129 cesarean sections                |
| <i>Risk factor</i>              |                               |                                      |                                      |
| Labor prior to cesarean section | 0.19 (1.0)                    | 1.14 (4.1)                           | 6.06 (1.8)                           |
| No. of vaginal examinations     | 0.28 (1.5)                    | 0.62 (2.2)                           | NS                                   |
| Time membranes ruptured         | 0.41 (2.2)                    | 0.74 (2.6)                           | NS                                   |
| Postoperative anemia            | 0.92 (4.8)                    | NS                                   | 6.57 (1.9)                           |
| Obesity                         | —                             | NS                                   | 7.42 (2.2)                           |
| Anesthesia                      | —                             | NS                                   | 17.56 (5.2)                          |
| Urgency of operation            | Significant                   | —                                    | 3.39 (1.0)                           |
| Duration or type of monitoring  | NS                            | 0.28 (1.0)                           | NS                                   |

Note: Raw score represents reported standardized discriminant coefficient or F-value, and number in brackets indicates relative weighting. NS = not statistically significant.

incidence of infection after emergency primary cesarean section (38.4%) than after nonurgent procedures (22.2%).

Since no statistically significant risk factors associated with endometritis after repeat cesarean section were identified, those factors contributing to the higher incidence of endometritis after primary cesarean section were investigated. Parity, month or day of operation, labor and delivery site used, obstetrician or resident performing the operation, and the duration of the operation were not found to be statistically significant. There was also no statistically significant difference between internal and external monitoring (Table II). However, a significantly increased risk ( $p < 0.01$ ) for development of endometritis after primary cesarean section was associated with four factors: duration of labor, number of preoperative vaginal examinations, time membranes were ruptured, and postoperative anemia (hemoglobin  $\leq 10.5$ ).

By means of multiple variant discriminant analysis (SPSS subroutine—Discriminant), the relative significance of each of the four aforementioned factors, which served as discriminant variables, was assessed. From the derived discriminant function, duration of labor was the least important risk factor, with a standardized discriminant coefficient of 0.19. For the other variables, the corresponding coefficients were number of vaginal examinations (0.28), time membranes were ruptured (0.41), and postoperative anemia (0.92). Thus, these variables were, respectively, 1.5, 2.2, and 4.8 times more important than duration of labor in predicting risk for subsequent endometritis. Of 15 patients who had a postoperative hemoglobin of less than 10.5, and membranes ruptured for over 4 hours after primary cesarean section, 10 (66.7%) subsequently developed endometritis. Interestingly, of 35 patients with a postoperative hemoglobin of  $<10.5$ , only four received transfusions (one for an abruptio placentae with

a disseminated intravascular coagulopathy, and three for significant postpartum hemorrhage). Of 10 patients with a hemoglobin of less than 9.0 who were subsequently treated with iron supplements, seven (70%) developed infections. However, only one patient had a preoperative hemoglobin of less than 10.5, and no patient received a transfusion because of excessive loss of blood during operation.

There was a significant difference ( $p < 0.05$ ) in bacteriologic flora isolated from uterine aspirates and high cervical cultures (when aspirate was unobtainable) for infections after primary and repeat cesarean section (Table III). *Staphylococcus aureus* was cultured from four patients (80%) after repeat cesarean section, including two with positive blood cultures. However, *Bacteroides* species were isolated in 13 patients (44.8%), and *S. aureus* in only five patients (17.2%), after primary cesarean section. A mixture of gram-negative anaerobic (most frequently *Bacteroides*) and aerobic organisms (most frequently *Escherichia coli*) occurred in nine cases (31.0%).

### Comment

This study attempted to identify the patient at risk for developing infection (in particular, endometritis) after cesarean section. Only patients who underwent primary cesarean section were found to be at increased risk of infection. There was no significant difference in infection rates after repeat cesarean section and after vaginal delivery, and no patient-related risk factors were identified. Rather, infection was attributable to poor sterile technique and contamination, as evidenced by the predominance of nosocomial organisms, such as *Staphylococcus aureus*, isolated on endometrial and blood cultures. These findings relate to attempts aimed at decreasing morbidity associated with cesarean section which will be discussed shortly.

In our series, four statistically significant risk factors



were identified as being associated with the occurrence of postoperative endometritis after primary cesarean section. These were, in decreasing order of significance, postoperative anemia, time membranes were ruptured prior to delivery, number of vaginal examinations, and duration of labor.

Anemic patients are prone to infection primarily because of decreased host resistance. Since no patient was anemic preoperatively or received a transfusion intraoperatively, this implies either an underestimation of operative loss of blood or a reluctance to subject patients to transfusions. However, because the risk of infection in such patients approaches 70%, further investigation is needed to determine whether accurate assessment of operative loss of blood with prompt replacement at the time of operation is warranted when the risk of transfusion reactions or hepatitis is less than 1% (as in our institution). The importance of proper antenatal nutrition and iron supplementation must also be emphasized since these can help compensate for operative loss of blood.

The other risk factors are interrelated and define the patient at highest risk preoperatively for developing postoperative endometritis, namely, one with ruptured membranes subjected to labor. In such a patient, infection is attributable to normal vaginal flora gaining access to the uterus because of ruptured membranes. Uterine ischemia during contractions associated with active labor, and tissue trauma associated with surgical procedures further predispose to infection. This is supported by three recent studies which assessed the relative significance of risk factors for postcesarean endometritis (Table IV). The duration of labor was found

to be significant in all studies. Ruptured membranes and the number of preoperative vaginal examinations were identified as risk factors in at least two studies. However, the factor determined to be most significant depended on the obstetric population and clinical practices and varied from institution to institution.

The importance of these findings relates to attempts aimed at decreasing morbidity associated with cesarean section. They can be summarized as follows: (1) Assessment of infectious complications after cesarean section necessitates precise identification of risk factors for both primary and repeat cesarean section at each individual institution. (2) The increased risk of infection in anemic patients must be recognized. Adequate antenatal nutrition, accurate assessment of operative loss of blood and, perhaps, prompt blood transfusion or antibiotic prophylaxis can benefit such patients. (3) Unless an increased risk for infection after repeat cesarean section can be demonstrated, antibiotic prophylaxis should be discouraged. Rather strict adherence to sterile technique can decrease infection without subjecting a patient to the risks associated with the administration of antibiotics. (4) Patients who undergo primary cesarean section after a prolonged labor with ruptured membranes or in an emergency situation are at greatest risk for developing postoperative endometritis. Such a high-risk group may benefit from antibiotic prophylaxis.

A prospective study to evaluate the effectiveness of antibiotic prophylaxis in selected patients at risk for infection after primary cesarean section is presently under way in our institution.

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# The prediction of intrapartum fetal metabolic acidosis by fetal heart rate monitoring

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Fetal heart rate characteristics during the 8 hours prior to delivery have been studied in 200 patients in whom the fetus had evidence of a metabolic acidosis at delivery, and compared to those in 200 patients in whom the fetus had a normal acid-base state at delivery. Baseline fetal heart rate moderate bradycardia and tachycardia, decreased baseline variability, and decreased fetal heart rate accelerations are not predictors of intrapartum fetal hypoxia with metabolic acidosis. Marked patterns of total decelerations and late decelerations are predictive of intrapartum fetal hypoxia with metabolic acidosis. The probability of fetal metabolic acidosis in the presence of a marked pattern of total decelerations is 25%, and with late decelerations it is 48%, in a population of high-risk pregnancies. (AM. J. OBSTET. GYNECOL. 139:299, 1981.)

THE VALUE OF electronic monitoring of fetal heart rate in the intrapartum surveillance of the fetus at risk in clinical practice continues to be questioned in spite of a growing body of knowledge of the relationship between abnormal fetal heart rate behavior and fetal hypoxemia.

The efficacy of a diagnostic procedure is a product of the predictive probability of the observations made for the specific question asked and the understanding of that probability by those responsible for the care of the patient. The purpose of the present study was to demonstrate the value of fetal heart rate characteristics in predicting the presence of a metabolic acidosis due to fetal hypoxia. The observations were derived from a representative group of high-risk patients in an obstetric unit serving the total population in a region.

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**Table I.** Clinical characteristics by which each patient in the control group was matched with a patient in the index group

|        |              | Index | Control |
|--------|--------------|-------|---------|
| Mother | Medical      |       |         |
|        | Normal       | 170   | 170     |
|        | Complication | 30    | 30      |
|        | Obstetric    |       |         |
| Labor  | Normal       | 111   | 111     |
|        | Complication | 89    | 89      |
|        | Labor        |       |         |
|        | Normal       | 120   | 120     |
|        | Abnormal     | 80    | 80      |
|        | Delivery     |       |         |
| Fetus  | Normal       | 69    | 69      |
|        | Abnormal     | 131   | 131     |
|        | Maturity     |       |         |
|        | <37 wk       | 20    | 20      |
|        | 37-42 wk     | 171   | 171     |
|        | >42 wk       | 9     | 9       |

## Methods

The population studied was derived from patients admitted to and monitored in the intrapartum intensive-care unit. Patients are admitted to the unit because of a risk factor of fetal asphyxia and represent approximately 15% of the patients cared for in this obstetric

**Table II.** Observations on mean baseline fetal heart rate and decrease in or absence of baseline variability in the index group compared with the control group in each of the 12 cycles during the 4 hours prior to delivery

| Hour before delivery | Cycle | No. of patients |         | Baseline fetal heart rate (Mean) |               |         | Baseline variability |               |         |             |               |         |
|----------------------|-------|-----------------|---------|----------------------------------|---------------|---------|----------------------|---------------|---------|-------------|---------------|---------|
|                      |       |                 |         |                                  |               |         | Decreased            |               |         | Absent      |               |         |
|                      |       | Index           | Control | Index (bpm)                      | Control (bpm) | P value | Index (No.)          | Control (No.) | P value | Index (No.) | Control (No.) | P value |
| 1                    | 1     | 200             | 200     | 145                              | 145           | 0.7     | 87                   | 95            | 0.5     | 26          | 34            | 0.3     |
|                      | 2     | 200             | 200     | 144                              | 144           | 0.8     | 99                   | 88            | 0.3     | 19          | 24            | 0.5     |
|                      | 3     | 200             | 200     | 143                              | 143           | 0.9     | 96                   | 94            | 0.9     | 21          | 28            | 0.4     |
| 2                    | 4     | 200             | 200     | 142                              | 142           | 0.5     | 94                   | 93            | 0.9     | 21          | 29            | 0.3     |
|                      | 5     | 200             | 200     | 142                              | 142           | 0.5     | 103                  | 88            | 0.2     | 21          | 25            | 0.6     |
|                      | 6     | 200             | 200     | 142                              | 142           | 0.9     | 95                   | 85            | 0.4     | 15          | 24            | 0.2     |
| 3                    | 7     | 170             | 172     | 141                              | 142           | 0.4     | 80                   | 83            | 0.9     | 12          | 21            | 0.1     |
|                      | 8     | 170             | 172     | 141                              | 141           | 0.7     | 81                   | 83            | 0.9     | 12          | 17            | 0.5     |
|                      | 9     | 170             | 172     | 140                              | 141           | 0.5     | 83                   | 76            | 0.4     | 9           | 17            | 0.2     |
| 4                    | 10    | 142             | 151     | 139                              | 141           | 0.4     | 68                   | 66            | 0.4     | 11          | 14            | 0.6     |
|                      | 11    | 142             | 151     | 140                              | 141           | 0.6     | 65                   | 64            | 0.6     | 8           | 13            | 0.6     |
|                      | 12    | 142             | 151     | 140                              | 141           | 0.7     | 64                   | 67            | 0.9     | 10          | 14            | 0.6     |

**Table III.** Observations on the decrease in or absence of fetal heart rate accelerations in the index group compared with the control group in each of the 12 cycles during the 4 hours prior to delivery

| Hour before delivery | Cycle | No. of patients |         | Fetal heart rate acceleration |               |         |             |               |         |
|----------------------|-------|-----------------|---------|-------------------------------|---------------|---------|-------------|---------------|---------|
|                      |       |                 |         | Decreased                     |               |         | Absent      |               |         |
|                      |       | Index           | Control | Index (No.)                   | Control (No.) | P value | Index (No.) | Control (No.) | P value |
| 1                    | 1     | 200             | 200     | 132                           | 128           | 0.4     | 89          | 80            | 0.4     |
|                      | 2     | 200             | 200     | 132                           | 133           | 0.9     | 98          | 77            | 0.04    |
|                      | 3     | 200             | 200     | 135                           | 130           | 0.4     | 98          | 89            | 0.4     |
| 2                    | 4     | 200             | 200     | 147                           | 132           | 0.2     | 99          | 85            | 0.2     |
|                      | 5     | 200             | 200     | 137                           | 131           | 0.4     | 97          | 89            | 0.5     |
|                      | 6     | 200             | 200     | 135                           | 124           | 0.09    | 108         | 90            | 0.09    |
| 3                    | 7     | 170             | 172     | 110                           | 113           | 0.9     | 70          | 70            | 0.9     |
|                      | 8     | 170             | 172     | 115                           | 114           | 0.9     | 87          | 71            | 0.08    |
|                      | 9     | 170             | 172     | 115                           | 113           | 0.9     | 82          | 77            | 0.6     |
| 4                    | 10    | 142             | 151     | 93                            | 100           | 0.9     | 61          | 68            | 0.8     |
|                      | 11    | 142             | 151     | 92                            | 93            | 0.6     | 57          | 60            | 0.9     |
|                      | 12    | 142             | 151     | 91                            | 98            | 0.9     | 67          | 68            | 0.8     |

unit. Continuous surveillance includes recording of uterine activity with an amniotic fluid catheter, electronic fetal heart rate monitoring with a scalp electrode, periodic fetal scalp blood acid-base assessment, and fetal cord blood acid-base assessment at delivery. A significant metabolic acidosis was an umbilical artery buffer base of  $<36.1$  mEq/L, which represents two standard deviations below the mean of an optimal population.

The study group included 200 patients in whom the fetus at delivery had an umbilical artery buffer base of  $<36.1$  mEq/L (hereafter referred to as the index group). The index group was matched with an additional 200 patients in whom the fetus at delivery had an umbilical artery buffer base of  $>38.6$  mEq/L (hereafter referred to as the control group). Each control case related to an index case and represented the next pa-

tient in time who matched the index case in respect to the presence or absence of major medical or obstetric complications, the occurrence of normal or abnormal labor and delivery, and was in the same fetal maturity category, that is,  $<37$  weeks, 37 to 42 weeks, or  $>42$  weeks (Table I).

The fetal heart rate records were scored for each 20 minute period for a maximum of 24 twenty-minute cycles (8 hours) prior to delivery. All records were scored by one of two authors (J. A. L. and M. J. C.) for uniformity of assessment. The records were scored without awareness of the clinical or laboratory data in order to avoid bias arising from a knowledge of the clinical circumstances.

The following fetal heart rate characteristics were scored in each 20 minute cycle of the fetal rate record.

**Baseline fetal heart rate.** This is the fetal heart rate



**Table IV.** Observations on the incidence of total decelerations, variable decelerations, and late decelerations in the index group compared with the control group in each of the 12 cycles during the 4 hours prior to delivery

| Hour before delivery | Cycle | No. of patients |         | Total decelerations (% deceleration) |         |         | Variable decelerations (% deceleration) |         |         | Late decelerations (No. with late) |         |         |
|----------------------|-------|-----------------|---------|--------------------------------------|---------|---------|-----------------------------------------|---------|---------|------------------------------------|---------|---------|
|                      |       | Index           | Control | Index                                | Control | P value | Index                                   | Control | P value | Index                              | Control | P value |
| 1                    | 1     | 200             | 200     | 51                                   | 33      | 0.0001  | 38                                      | 30      | 0.01    | 78                                 | 23      | 0.001   |
|                      | 2     | 200             | 200     | 42                                   | 30      | 0.0001  | 30                                      | 26      | 0.2     | 59                                 | 21      | 0.001   |
|                      | 3     | 200             | 200     | 35                                   | 26      | 0.006   | 26                                      | 24      | 0.3     | 42                                 | 21      | 0.01    |
| 2                    | 4     | 200             | 200     | 28                                   | 26      | 0.4     | 22                                      | 23      | 0.8     | 38                                 | 23      | 0.05    |
|                      | 5     | 200             | 200     | 28                                   | 22      | 0.03    | 23                                      | 18      | 0.1     | 32                                 | 17      | 0.03    |
|                      | 6     | 200             | 200     | 24                                   | 23      | 0.8     | 21                                      | 21      | 0.9     | 28                                 | 16      | 0.08    |
| 3                    | 7     | 170             | 172     | 22                                   | 23      | 0.8     | 20                                      | 21      | 0.6     | 19                                 | 12      | 0.2     |
|                      | 8     | 170             | 172     | 23                                   | 19      | 0.2     | 19                                      | 17      | 0.6     | 22                                 | 11      | 0.06    |
|                      | 9     | 170             | 172     | 18                                   | 20      | 0.7     | 16                                      | 18      | 0.5     | 20                                 | 10      | 0.08    |
| 4                    | 10    | 142             | 151     | 19                                   | 19      | 0.9     | 17                                      | 16      | 0.7     | 11                                 | 12      | 0.8     |
|                      | 11    | 142             | 151     | 18                                   | 18      | 0.9     | 17                                      | 17      | 0.9     | 11                                 | 5       | 0.2     |
|                      | 12    | 142             | 151     | 20                                   | 15      | 0.1     | 17                                      | 14      | 0.3     | 12                                 | 6       | 0.2     |

in the interval between periodic heart rate changes in relation to contractions. Baseline fetal heart rate was classified as normal (120 to 160 bpm), bradycardia (<120 bpm), and tachycardia (>160 bpm).

**Baseline fetal heart rate long-term variability.** This represents the more or less irregular oscillations or fluctuations of the recorded fetal heart rate. Baseline variability was classified on the amplitude of oscillation as normal (6 to 25 bpm), decreased (3 to 5 bpm), and absent (<3 bpm).

**Fetal heart rate accelerations.** Accelerations are short-term increases in fetal heart rate of at least 15 bpm above the baseline fetal heart rate. Accelerations may be either sporadic, not related to contractions, or periodic, with contractions. Accelerations were classified as normal ( $\geq 2$  accelerations in 20 minutes), decreased (one acceleration in 20 minutes), and absent (no accelerations in 20 minutes).

**Total fetal heart rate decelerations.** A deceleration is a transitory fall in fetal heart rate in excess of 15 bpm from a given baseline in relation to a contraction. The percentage of contractions associated with a deceleration was established in each 20 minute period. Total deceleration patterns were classified on the basis of the frequency of contractions in each 20 minute period: none (0% to 4% contractions associated with a deceleration), moderate (5% to 30% contractions associated with a deceleration), marked (>30% contractions associated with a deceleration).

**Fetal heart rate variable decelerations.** Variable decelerations vary markedly in shape from contraction to contraction. The departure and return of the fetal heart rate are abrupt and the lag time varies.

**Fetal heart rate late decelerations.** Late decelerations are characterized by an onset late in the contraction phase, and the maximum point of the deceleration

occurs more than 20 seconds after the acme of the contraction.

## Results

The observations on baseline fetal heart rate and baseline variability in the index group were compared with those in the control group in each of the 24 cycles during the 8 hours of labor prior to delivery. The data for 4 hours prior to delivery are presented in Table II. There was no statistical difference between the two groups in respect to either the mean baseline fetal heart rate or the frequency of bradycardia or tachycardia. Similarly, in regard to baseline variability, there was no statistical difference between the two groups in respect to either decreased or absent baseline variability. These observations imply that, as independent variables, baseline fetal heart rate (specifically, baseline bradycardia or baseline tachycardia) and baseline long-term variability (specifically, decreased variability) are not predictive of metabolic acidosis in the fetus.

The observations on fetal heart rate accelerations in the index group were compared with those in the control group in each of the 24 cycles during the 8 hours of labor prior to delivery. The data for the 4 hours prior to delivery are presented in Table III. There was no statistical difference between the two groups in regard to either decreased frequency of absence of fetal heart rate accelerations. These observations imply that, as an independent variable, fetal heart rate accelerations (specifically, a decrease in or absence of accelerations) are not predictive of metabolic acidosis in the fetus.

The observations on total fetal heart rate decelerations in the index group were compared with those in the control group in each of the 24 cycles during the 8 hours prior to delivery. The data for the 4 hours prior to delivery are presented in Table IV. The percentage

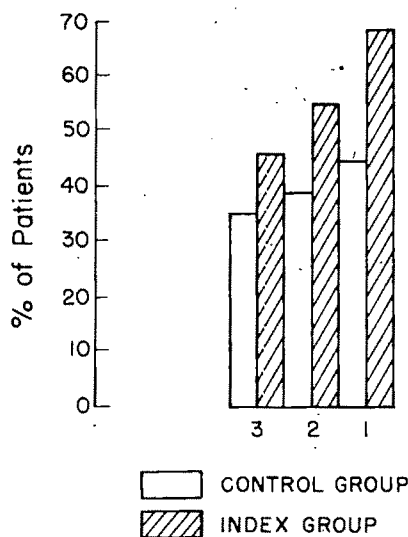


Fig. 1. The incidence of marked patterns of total decelerations in the index group and control group for the three cycles during the hour prior to delivery.

of contractions associated with a deceleration was significantly greater in the index group than in the control group in cycles 1, 2, and 3. This difference was associated with a greater frequency of marked patterns of total decelerations in the index group than in the control group in cycle 1 (68% vs. 44%), cycle 2 (55% vs. 38%), and cycle 3 (46% vs. 35%). These observations imply that, as an independent variable, total fetal heart rate decelerations (specifically, marked patterns of total decelerations) are predictive of metabolic acidosis.

The observations on fetal heart rate variable decelerations in the index group were compared with those in the control group in each of the 24 cycles during the 8 hours prior to delivery. The data for the 4 hours prior to delivery are presented in Table IV. The percentage of contractions associated with a variable deceleration was significantly greater in the index group than in the control group in cycle 1; however, thereafter, there was no statistical difference between the two groups. These observations imply that, as an independent variable, fetal heart rate variable decelerations are of limited value in predicting metabolic acidosis in the fetus, which is best expressed through total decelerations.

The observations on fetal heart rate late decelerations in the index group were compared with those in the control group in each of the 24 cycles during the 8 hours prior to delivery. The data for the 4 hours prior to delivery are presented in Table IV. The number of fetuses with late decelerations was significantly greater in the index group than in the control group in cycles

1, 2, and 3. Subsequently, there was a trend for the number in the index group to be greater than that in the control group in cycles 4, 5, and 6. These observations imply that, as an independent variable, fetal heart rate late decelerations are predictive of metabolic acidosis in the fetus.

Although baseline fetal heart rate and baseline fetal heart rate long-term variability did not prove to be of predictive value as independent variables, it is appropriate to assess the significance of these observations in conjunction with either total fetal heart rate decelerations or fetal heart rate late decelerations. Statistical analysis of the predictive value of these combined observations failed to demonstrate that either baseline fetal heart rate bradycardia or tachycardia or baseline fetal heart rate decreased or absent variability significantly increased the predictive value of either total fetal heart decelerations or fetal heart rate late decelerations for metabolic acidosis in the fetus.

#### Comment

Much of the confusion in the assessment of the value of fetal heart rate surveillance is due to the differing questions asked and endpoints used in the studies reported. There are three stages in the pathophysiologic process of fetal hypoxia, each of which is important and requires accurate diagnosis in the human fetus to permit optimal clinical management. These stages are transient hypoxemia without fetal metabolic acidosis, fetal hypoxemia and tissue hypoxia sufficient to produce a metabolic acidosis, and, finally, fetal hypoxia with metabolic acidosis of a degree and duration sufficient to cause fetal morbidity or mortality.

During labor and delivery, transient hypoxemia without metabolic acidosis may be identified by measurement of fetal oxygen tension from an appropriately timed sample of scalp blood or by the recording of a continuous oxygen tension electrode. Identification of a transient fall in oxygen tension is useful in that it may affect fetal behavior and provide the first indication of a developing mechanism which, if allowed to continue, will result in a more serious degree of fetal intrapartum hypoxia.

The diagnosis of fetal hypoxia with a metabolic acidosis requires an acid-base assessment of fetal blood. The specific aim in this study was to demonstrate the predictive value of continuously recorded fetal heart rate observations of fetal hypoxia with a metabolic acidosis with the endpoint a metabolic acidosis in the umbilical artery blood at delivery. The limited correlation between fetal metabolic acidosis and immediate and long-term morbidity commonly reported is not surprising and, indeed, is desirable, thereby indicating that



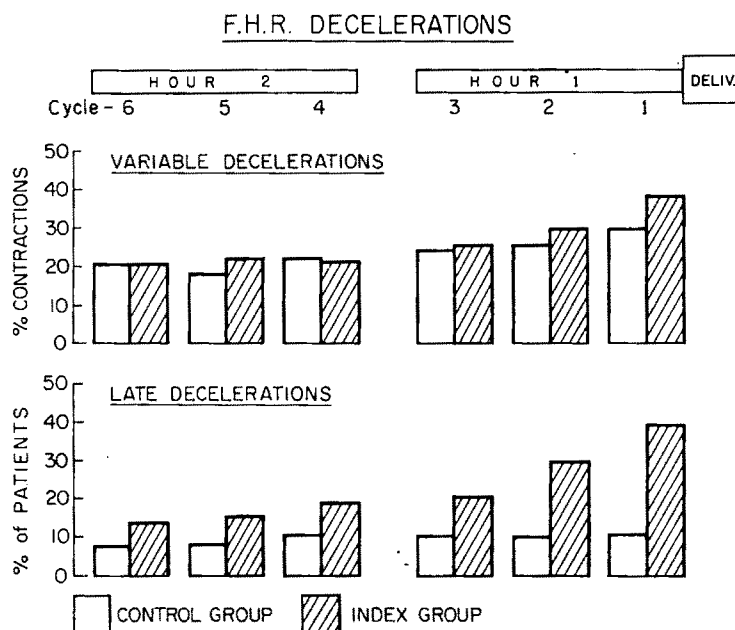


**Fig. 2.** The incidence of variable decelerations and late decelerations in the index group and control group for the six cycles during the 2 hours prior to delivery.

the fetal hypoxia has been identified at an early stage before fetal compromise has occurred. On the other hand, these are the at-risk infants in whom morbidity or mortality will result if the pathophysiologic process is allowed to continue.<sup>1</sup>

The present study indicates that fetal heart rate bradycardia or tachycardia, a decrease in or absence of baseline variability, and a decrease in or absence of fetal heart rate accelerations are not predictors of intrapartum fetal hypoxia with a metabolic acidosis. We emphasized that this does not imply that the mechanisms which lead to these changes in fetal heart rate behavior may not be relevant to the occurrence of subsequent morbidity and, as such, have predictive significance in regard to the ultimate outcome of the newborn infant.

Severe fetal heart rate bradycardia, <100 bpm, has been observed terminally prior to fetal death,<sup>2</sup> and has been stated to indicate a poor outcome. This observation is uncommon, with occurrence in this study on one occasion in a fetus in the control group. Moderate fetal heart rate bradycardia, 100 to 119 bpm, occurred with equal frequency in the index and control groups, which is in keeping with the recent reports of normal fetal pH in relation to moderate bradycardia.<sup>3</sup>

Examples of tachycardia in the dying fetus suggested the possible significance of fetal heart rate tachycardia in respect to fetal tachycardia in acute hypoxia studies of the fetus in anesthetized sheep,<sup>4</sup> a response which

is now attributed to the anesthetic suppression of parasympathetic regulation of fetal heart rate. The occurrence of fetal heart rate tachycardia with equal frequency in the index and control groups is in keeping with previous observations of no significant relationship between fetal tachycardia and metabolic acidosis.<sup>5</sup>

A number of observations suggested the possible significance of decreased fetal heart rate long-term variability in the prediction of intrapartum fetal hypoxia. A decrease in or loss of baseline variability during the antepartum period has been identified as a predictor of potential fetal compromise. Intrapartum observations of the association of decreased baseline variability in the severely compromised fetus led to the conclusion that this was indicative of severe distress and a poor prognosis. However, recent acid-base correlations indicate that, with decreased variability, although the pH may be lower, the difference is small.<sup>6, 7</sup> In view of these reported acid-base relationships, it is not surprising that the present study indicated that decreased or absent baseline variability in the intrapartum period may occur with equal frequency in the fetus with and in the fetus without metabolic acidosis.

Accelerations indicate sympathetic responsiveness and a good prognosis<sup>2</sup> and exhibit a positive correlation to a good neonatal outcome.<sup>8</sup> On the other hand, there has been little evidence to indicate that a decrease in or absence of accelerations is either correlated with or predictive of intrapartum fetal hypoxia. The present

**Table V.** Probability of metabolic acidosis in relation to the scoring system in a general and high-risk obstetric population

| Score | Criteria                                | General population (%) | High-risk population (%) |
|-------|-----------------------------------------|------------------------|--------------------------|
| 0     | No decelerations                        | 6                      | 17                       |
| 1     | Moderate pattern of total decelerations | 7                      | 19                       |
| 2     | Marked pattern of total decelerations   | 10                     | 25                       |
| 3     | Late decelerations                      | 25                     | 48                       |

study indicated that a decrease in or absence of accelerations may occur with equal frequency during the intrapartum period in the fetus with and in the fetus without metabolic acidosis.

The significant findings are that fetal heart rate decelerations, specifically, marked patterns of total decelerations and late decelerations, are useful predictors of intrapartum fetal hypoxia with metabolic acidosis.

Total decelerations represents the sum of variable and late decelerations. The observation of total decelerations in clinical practice is useful in that, although there may be differences of opinion from time to time in regard to the classification of the type of a deceleration, the recognition of the presence of a deceleration is not difficult and rarely controversial. The significant observation is a marked pattern of total decelerations, i.e., >30% of contractions are associated with a deceleration. The incidence of marked patterns of total decelerations in the index group was significantly greater than that in the control group for the three cycles during the hour prior to delivery (Fig. 1).

Variable decelerations are due to a reflex parasympathetic stimulation via the vagus nerves. This mechanism has been demonstrated in animal studies<sup>9</sup> and clinical studies of the human fetus.<sup>10</sup> Variable decelerations occur in the presence of a normal fetal acid-base state.<sup>11</sup>

In the present study, variable decelerations occurred with greater frequency in the index group than in the control group during the last cycle prior to delivery, but, otherwise, variable decelerations occurred with equal frequency during the intrapartum period in the fetus with and in the fetus without metabolic acidosis (Fig. 2).

There is evidence that, as the frequency of variable decelerations increases, a correlation with fetal hypoxia may appear. This might be anticipated in view of the relationship between cardiac rate and cardiac output in the fetus,<sup>12</sup> and is in keeping with the observation of a significant relationship between the index and control

groups for variable decelerations during the 20 minutes prior to delivery and total decelerations during the hour prior to delivery.

The importance of fetal hypoxia in the occurrence of late decelerations has been extensively demonstrated. Studies in animals have shown that late decelerations occur when fetal oxygen tension decreases below a critical level, and the interval between the onset of the contraction and the onset of the deceleration reflects the time necessary for the fetal oxygen tension to fall below the tolerance level.<sup>13, 14</sup> The late deceleration is not a response to the pH change of acidosis.<sup>15</sup> Fetal hypoxemia results in a late deceleration due either to a chemoreceptor-initiated reflex bradycardia or to a direct effect upon the myocardium.<sup>16</sup> Studies in the human fetus have similarly demonstrated the relationship between metabolic acidosis and late decelerations.<sup>5-7, 17, 18</sup>

The observations in the present study are in keeping with those of previous reports. Late decelerations occur with greater frequency in the fetus with metabolic acidosis than in the fetus with no metabolic acidosis in the three cycles during the hour prior to delivery, with a trend toward a greater frequency in the three cycles during the second hour prior to delivery (Fig. 2).

From the data derived in this study, a scoring system was designed to estimate the predictive value of fetal heart rate decelerations for the presence of a significant degree of metabolic acidosis. It was assumed that the incidence of metabolic acidosis in a typical general population was of the order of 10%, and that in a typical high-risk population was of the order of 25%, on the basis of the experience in this unit during the last decade. The probability of metabolic acidosis increased in relation to this scoring system, so that with late decelerations the probability of metabolic acidosis is 23% in the general population and 48% in the high-risk population (Table V).

Continuous electronic monitoring of fetal heart rate is a useful complement to acid-base assessment in the intrapartum surveillance of the fetus in a high-risk pregnancy. The appearance of a marked pattern of total decelerations and, particularly, late decelerations is associated with a very significant probability of fetal metabolic acidosis and indicates the need for an acid-base assessment. However, the results of this study again serve to emphasize that, at some stage, the fetus in all at-risk pregnancies should have an acid-base assessment, in view of the 17% of high-risk patients in whom the fetus exhibits evidence of a significant metabolic acidosis with no preceding fetal heart rate decelerations.



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# The effect of maternal, labor, and fetal factors upon fetal heart rate during the intrapartum period

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The effect of maternal, labor, and fetal characteristics upon fetal heart rate behavior during the intrapartum period has been studied in 400 patients. Abnormal labor in comparison to normal labor has a higher baseline fetal heart rate with an increased incidence of baseline tachycardia and an increased incidence of absent or decreased baseline variability. A decreasing fetal weight gestational age percentile is associated with an increased incidence of variable decelerations. Segmental epidural and Demerol analgesia carefully administered has little effect upon fetal heart rate behavior. (AM. J. OBSTET. GYNECOL. 139:306, 1981.)

THIS STUDY was undertaken to demonstrate the effect of maternal, labor, and fetal complications upon fetal heart rate behavior. An increasing number of factors is recognized as being potentially capable, through autonomic nervous system activity, of altering fetal heart rate behavior. To understand the effect of factors other than fetal hypoxia upon fetal heart rate will enhance our ability to interpret fetal heart rate observations during labor.

## Methods

The study group included 400 patients, 200 with and 200 without evidence of fetal metabolic acidosis at delivery in whom the fetal heart rate records were scored for each 20 minute period for a maximum of 8 hours (24 twenty-minute cycles) prior to delivery. The fetal heart rate characteristics assessed included baseline fetal heart rate, baseline fetal heart rate long-term variability, fetal heart rate accelerations, and fetal heart

rate variable and late decelerations according to the criteria previously described.<sup>1</sup>

The clinical characteristics of each patient, including maternal, medical, and obstetric complications, labor and delivery characteristics, and fetal characteristics were defined, coded, and stored for statistical analysis (Table I). The significance of each clinical characteristic in relation to each fetal heart rate characteristic studied was analyzed by means of Student's t test or chi-square test.

The patients received a number of drugs during labor. The drugs given in each cycle were noted, and the changes in the fetal heart rate in the cycle after administration of the drug in relation to the prior cycle were observed in order to evaluate the drug's effect (Fig. 1). The changes noted in the sequence of cycles related to the drug administered were compared to the findings in the sequence of cycles in which the drug was not administered.

## Results

Fetal heart rate behavior in respect to baseline fetal heart rate, baseline fetal heart rate variability, fetal heart rate accelerations, or fetal heart rate decelerations was the same in patients with as in those without medical complications and in patients with and those without obstetric complications.

There was a relationship between abnormal labor and baseline fetal heart rate and baseline fetal heart

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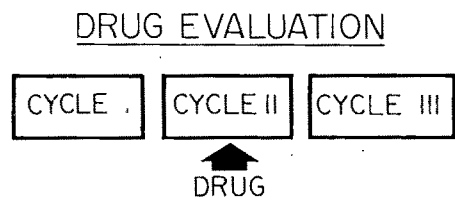


**Fig. 1.** The effect of the drug administered in cycle 2 was assessed by the changes in fetal heart rate behavior in cycle 3 in relation to cycle 1.

**Table I.** Maternal, labor, and fetal characteristics defined in each patient

|          |                                       |                                    |
|----------|---------------------------------------|------------------------------------|
| Maternal | Medical                               | Obstetric                          |
|          | Normal                                | Normal                             |
|          | Diabetic                              | Toxemia                            |
|          | Cardiac                               | Antepartum hemorrhage              |
|          | Hypertension                          | Toxemia plus antepartum hemorrhage |
|          | Other                                 | Other                              |
| Labor    | Labor                                 | Delivery                           |
|          | Normal                                | Normal                             |
|          | Abnormal uterine action               | Abnormal forceps                   |
|          | Dystocia                              | Breech                             |
|          | Abnormal uterine action plus dystocia | Cesarean section                   |
| Fetal    | Other                                 | Other                              |
|          | Gestational age                       |                                    |
|          | Birth weight                          |                                    |
|          | Weight gestational age percentile     |                                    |

rate variability for the 3 hours prior to delivery (Table II). The mean baseline fetal heart rate was significantly higher in patients with abnormal labor than in patients with normal labor in cycles 1 to 9. Similarly, the incidence of decreased or absent baseline fetal heart rate variability was greater in patients with abnormal labor than in patients with normal labor in cycles 1 to 9. No relationship was demonstrated between abnormal labor and fetal heart rate accelerations or fetal heart rate decelerations.

Fetal heart rate behavior in respect to baseline fetal heart rate, baseline fetal heart rate variability, fetal heart rate accelerations, and fetal heart rate decelerations was the same in patients who required an abnormal delivery as in those who had a normal delivery.

Fetal heart rate behavior in respect to baseline fetal heart rate, baseline variability, and fetal heart rate accelerations was the same over the range of gestational age, fetal weight, and fetal weight gestational age percentile in this study population. However, there was a relationship between fetal weight gestational age percentile and the frequency of variable decelerations (Table III). A significant correlation existed between decreasing fetal weight gestational age percentile and an

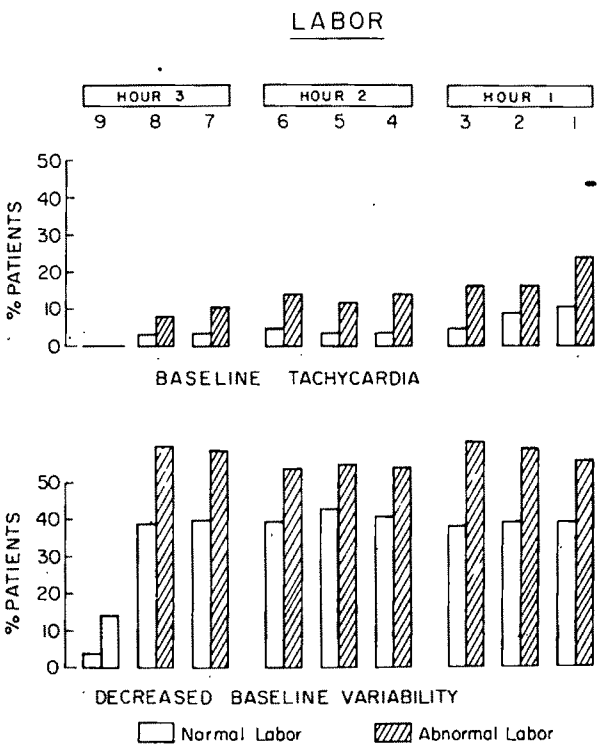


**Fig. 2.** The incidence of baseline fetal heart rate tachycardia and absent or decreased baseline variability in abnormal labor as compared to normal labor.

increasing percentage of contractions associated with a variable deceleration. No relationship was found between fetal weight gestational age percentile and either the occurrence or frequency of late decelerations.

Segmental epidural analgesia, usually with 5 to 7 ml of 0.25% or 0.50% bupivacaine, was initiated or reinforced on 841 occasions in the study population. The response to the epidural analgesia was the same as in the control cycles in respect to baseline fetal heart rate and baseline variability. There was a difference in regard to the number of accelerations, with a less frequent increase in the number of accelerations after epidural analgesia than in the control cycles ( $p < 0.01$ ) (Table IV).

Demerol, usually 15 mg intravenously, was given on 351 occasions. A difference was noticed in regard to baseline fetal heart rate and baseline variability after the administration of Demerol, with a less frequent increase in baseline fetal heart rate ( $p < 0.01$ ) and in baseline variability ( $p < 0.01$ ). The sequence of events in regard to the number of accelerations with Demerol was the same as in the cycles with no Demerol.

**Comment**

As labor progresses to delivery, a small but significant rise in baseline fetal heart rate occurs.<sup>2</sup> Ab-

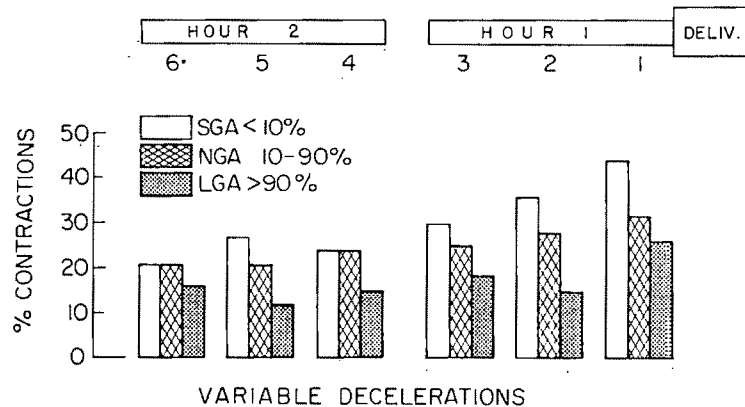


Fig. 3. The incidence of variable decelerations in the SGA, NGA, and LGA groups during the 2 hours prior to delivery.

Table II. Mean baseline fetal heart rate and incidence of decreased and absent baseline variability in abnormal labor compared to normal labor during the 3 hours prior to delivery

| Hour | Cycle | No. of patients |                | Baseline fetal heart rate |                             |         | Baseline variability |                      |         |                    |                      |         |
|------|-------|-----------------|----------------|---------------------------|-----------------------------|---------|----------------------|----------------------|---------|--------------------|----------------------|---------|
|      |       |                 |                |                           |                             |         | Decreased            |                      |         | Absent             |                      |         |
|      |       | Normal labor    | Abnormal labor | Normal labor (mean) (bpm) | Abnormal labor (mean) (bpm) | P value | Normal labor (No.)   | Abnormal labor (No.) | P value | Normal labor (No.) | Abnormal labor (No.) | P value |
| 1    | 1     | 238             | 162            | 142                       | 149                         | 0.0001  | 92                   | 90                   | 0.001   | 26                 | 34                   | 0.01    |
|      | 2     | 238             | 162            | 141                       | 148                         | 0.0001  | 92                   | 95                   | 0.0001  | 13                 | 30                   | 0.0001  |
|      | 3     | 238             | 162            | 141                       | 147                         | 0.0001  | 91                   | 99                   | 0.0001  | 14                 | 35                   | 0.0001  |
| 2    | 4     | 238             | 162            | 140                       | 146                         | 0.0001  | 99                   | 88                   | 0.01    | 21                 | 29                   | 0.01    |
|      | 5     | 238             | 162            | 140                       | 146                         | 0.0001  | 102                  | 89                   | 0.02    | 19                 | 27                   | 0.01    |
|      | 6     | 238             | 162            | 139                       | 145                         | 0.0001  | 93                   | 87                   | 0.01    | 17                 | 22                   | 0.05    |
| 3    | 7     | 195             | 147            | 140                       | 144                         | 0.01    | 78                   | 85                   | 0.001   | 10                 | 23                   | 0.01    |
|      | 8     | 195             | 147            | 139                       | 143                         | 0.01    | 76                   | 88                   | 0.001   | 9                  | 20                   | 0.01    |
|      | 9     | 195             | 147            | 139                       | 143                         | 0.01    | 76                   | 87                   | 0.001   | 6                  | 20                   | 0.01    |

Table III. Correlation between fetal weight gestational age percentile and the percentage of contractions associated with a variable deceleration in each cycle during the 2 hours prior to delivery

| Hour | Cycle | Correlative coefficient | P value |
|------|-------|-------------------------|---------|
| 1    | 1     | -0.23                   | 0.0001  |
|      | 2     | -0.24                   | 0.0001  |
|      | 3     | -0.14                   | 0.001   |
| 2    | 4     | -0.10                   | 0.02    |
|      | 5     | -0.12                   | 0.01    |
|      | 6     | -0.06                   | 0.1     |

normal labor in comparison to normal labor is characterized by a significantly higher baseline fetal heart rate during the 3 hours prior to delivery. Another difference between the abnormal and normal labor groups is demonstrated by the twice as frequent occurrence of baseline fetal heart rate tachycardia in patients with abnormal labor (Fig. 2).

Baseline fetal heart rate is a result of spontaneous fetal heart rate modified by the tonic effect of sympathetic and parasympathetic activity. Acceleration may occur because of an increase in beta adrenergic sympathetic effect,<sup>3</sup> or because of blockade of the tonic parasympathetic effect.<sup>4, 5</sup> Labor is characterized by an increase in circulating catecholamines,<sup>6</sup> which may account for part of this response overcoming the inhibition by parasympathetic efferent vagal nerves.

Abnormal labor in comparison to normal labor also demonstrates a greater incidence of absent and decreased baseline variability. Absence of baseline variability occurs twice as frequently, and decreased variability one and one-half times as frequently, in abnormal as in normal labor during the 3 hours prior to delivery (Fig. 2).

Baseline variability reflects moment-to-moment differences in parasympathetic cardiac regulatory activity due to a variety of stimuli, such as intravascular pres-



**Table IV.** Fetal heart rate behavior in regard to baseline fetal heart rate, baseline variability, and fetal heart rate accelerations in the sequence of cycles prior to and after the administration of epidural analgesia and Demerol compared to that in the sequence of cycles in which no analgesia was given

|                       | Baseline fetal heart rate |               |              |         | Baseline variability |               |              |         | Fetal heart rate acceleration |               |              |         |
|-----------------------|---------------------------|---------------|--------------|---------|----------------------|---------------|--------------|---------|-------------------------------|---------------|--------------|---------|
|                       | Increase (%)              | No change (%) | Decrease (%) | P value | Increase (%)         | No change (%) | Decrease (%) | P value | Increase (%)                  | No change (%) | Decrease (%) | P value |
| Epidural analgesia    | 36                        | 36            | 27           | <0.3    | 17                   | 63            | 20           | <0.8    | 26                            | 42            | 32           | <0.01   |
| No epidural analgesia | 34                        | 36            | 30           |         | 18                   | 62            | 20           |         | 31                            | 37            | 32           |         |
| Demerol               | 25                        | 40            | 35           | <0.01   | 12                   | 68            | 20           | <0.01   | 30                            | 33            | 37           | <0.1    |
| No Demerol            | 34                        | 36            | 30           |         | 18                   | 62            | 20           |         | 31                            | 37            | 32           |         |

sure, intrapleural pressure, and fetal movements.<sup>7, 8</sup> Abnormal labor would appear to suppress this regulatory activity to a greater degree than does normal labor.

Fetal weight gestational age, as previously reported,<sup>9</sup> is relevant to the occurrence of variable decelerations, with a significant correlation during the 2 hours prior to delivery between decreasing weight gestational age percentile and an increasing incidence of variable decelerations. This relationship for clinical purposes can be expressed by the frequency of variable decelerations in the small-for-gestational age (SGA), normal-for-gestational age (NGA), and large-for-gestational age (LGA) groups (Fig. 3). Because of this increased incidence of variable decelerations, marked patterns of total decelerations occur twice as frequently in the SGA groups compared to the LGA group. This suggests that stimuli which initiate reflex parasympathetic bradycardia occur with greater frequency in the small growth-retarded fetus.

The epidural analgesia used extensively in the management of the patients during labor appeared to have no effect other than to cause a less frequent increase in the number of accelerations in the subsequent fetal heart rate cycle (Fig. 4). Significant concentrations of local anesthetic in both the mother and fetus occur after administration,<sup>10</sup> with variation in concentration related to the dosage and the technique used. Maternally administered local anesthetic agents produce a transient uterine vasoconstriction and reduced uterine blood flow,<sup>11</sup> and a transient decrease in fetal oxygen tension without acidosis.<sup>12</sup> This effect may be associated with a transient fetal bradycardia in animals,<sup>12</sup> and increased baseline variability in the human fetus.<sup>13</sup> The absence of any significant effect is likely to be a result of the segmental block epidural analgesia with small volumes of local anesthetic agent, which, as previously

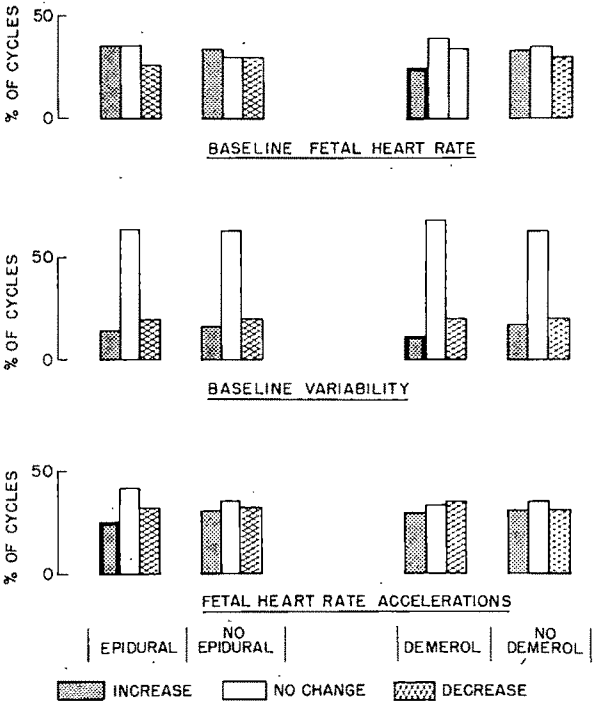


**Fig. 4.** The effect of epidural analgesia and Demerol upon baseline fetal heart rate, baseline variability, and fetal heart rate accelerations.

demonstrated, avoids much of the apparent fetal heart rate effect noticed in earlier studies.<sup>14</sup>

An increase in baseline fetal heart rate and in baseline variability occurred less frequently in cycles after the administration of Demerol than in the control cycles (Fig. 4). A small, but not statistically significant, decreased baseline variability has been reported in 18 patients who received 50 mg of Demerol intravenously.<sup>15</sup> The limited apparent effect in these patients may have been due to the use of 15 mg intravenously.

The results in regard to epidural analgesia and Demerol indicate that these analgesic agents, when carefully used, have little effect upon fetal heart rate behavior either in the fetus with or in the fetus without

metabolic acidosis. The careful use of these analgesics in the management of the at-risk patient during the intrapartum period is justified.

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# Squamous cell carcinoma of the vagina following hysterectomy

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A retrospective analysis of 29 cases of squamous cell neoplasia of the vagina following hysterectomy is presented. Two groups of patients were identified; the first presented following hysterectomy for cervical dysplasia or neoplasia on an average of 5.7 years later, and the second presented following hysterectomy for benign or unrelated disease 13.1 years after initial surgery. All 17 patients in the first group had Stage I disease whereas two thirds of the second group had Stage II or greater disease at the time of diagnosis. Surgery was the primary mode of therapy in 82.4% of the first group and radiotherapy was used in 83.3% of the second group. In all patients 20.7% had received previous radiotherapy to the pelvis. Carcinoma of the vagina following hysterectomy for benign disease tends to be more advanced at the time of diagnosis and subsequently has a poorer ultimate prognosis. Disease following previous cervical neoplasia is often asymptomatic and only detected on routine cytologic testing. (AM. J. OBSTET. GYNECOL. 139:311, 1981.)

SQUAMOUS CELL NEOPLASIA of the vagina following hysterectomy has been reported to occur subsequent to surgery for both benign and malignant disease. The most common predisposing factor is previous treatment for cervical dysplasia or neoplasia. Funnell and Merrill<sup>1</sup> found the recurrence rate of in-situ carcinoma in the vagina following treatment for in-situ disease of the cervix to be 0.7% to 6.6%. This may represent persistent or residual disease from the initial lesion or else a new lesion if occurring 1 year or more after surgery. Vaginal neoplasia following surgery for benign disease has been alluded to by Schiffer and associates,<sup>2</sup> Gallup and Morley,<sup>3</sup> and Rutledge,<sup>4</sup> but it continues to be associated with a delay in diagnosis. Patients with a previous benign abnormality tend to present with more advanced lesions and have poorer ultimate prognosis.

Twenty-nine cases of vaginal carcinoma following hysterectomy were diagnosed and treated at Victoria Hospital between 1969 and 1978. It is the purpose of

this paper to emphasize the need for continuous cytologic surveillance in posthysterectomy patients following both benign and malignant disease. Patients with previously treated benign lesions tend to present later and have a poorer prognosis than those with a previous malignancy. Previous pelvic irradiation may be a significant factor in the development of vaginal carcinoma.

## Material and Methods

Twenty-nine patients with neoplastic disease of the vagina were seen at Victoria Hospital in a 10-year period. Age at the time of diagnosis of vaginal carcinoma ranged from 33 to 86 years. All but five patients were multiparous. Four patients (13.8%) were premenopausal and the remainder (86.2%) were postmenopausal. Four patients (13.8%) were noted to be on a regimen of hormone replacement therapy at the time of diagnosis. Diagnosis of the vaginal lesion was made on the basis of a cytologic smear and subsequent biopsy in 11 patients (38%) and by biopsy of a visible lesion in 47 patients (59%). One patient had the diagnosis made at the time of laparotomy for a pelvic mass.

Seventeen patients in group 1 presented following operation for malignant disease and were compared to 12 patients in group 2 who had previous benign disease.

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**Table I.** Indications for hysterectomy in patients with previous cervical neoplasia (group 1)

| <i>Cervical neoplasia</i> | <i>No.</i> |
|---------------------------|------------|
| Dysplasia                 | 4          |
| In-situ                   | 8          |
| Stage I                   | 2          |
| Stage IIA                 | 2          |
| Recurrence                | 1          |

**Table II.** Indications for hysterectomy in patients with previous benign or unrelated disease (group 2)

| <i>Indication</i>                     | <i>No.</i> |
|---------------------------------------|------------|
| Uterine fibroids                      | 2          |
| Uterine prolapse                      | 2          |
| Pelvic inflammatory disease           | 1          |
| Stage I adenocarcinoma of endometrium | 2          |
| Unknown                               | 5          |

### Results

Patients with previous malignant disease presented 1 to 22 years after initial surgery with an average of 5.7 years. Those with prior benign disease presented 1 to 30 years following hysterectomy with an average of 13.1 years. In the first group the indications for hysterectomy are noted in Table I. The one patient with a recurrence underwent irradiation for carcinoma of the cervix 15 years earlier and developed a recurrence, treated by radical hysterectomy 2 years before this presentation. In the second group, the indications are summarized in Table II. The two patients with previous adenocarcinoma of the endometrium had well-differentiated Stage I lesions at 5 and 21 years, respectively, before this presentation and were treated with intrauterine radium and total hysterectomy.

Of all 29 patients, six had a history of previous pelvic irradiation. Four of these were treated for cervical neoplasia and two for endometrial adenocarcinoma. The interval between the radiation and the diagnosis of vaginal neoplasia averaged 14 years (range 5 to 22 years).

The presentation of the patients in the two groups is compared in Table III. The large majority of patients with previous malignant disease were usually examined cytologically and a new lesion was asymptomatic. Patients with benign disease did not present unless there was an abnormal symptom such as bleeding. Some patients had more than one presenting complaint.

The pathology of the vaginal lesion is presented in Table IV. The preponderance of early lesions, including severe dysplasia and carcinoma in situ, occurred in the first group of patients, reflecting the more careful

**Table III.** Mode of presentation of patient

| <i>Presentation</i>  | <i>Previous neoplasia</i> | <i>Previously benign or unrelated disease</i> |
|----------------------|---------------------------|-----------------------------------------------|
| Asymptomatic         | 10                        | 2                                             |
| Vaginal bleeding     | 4                         | 10                                            |
| Vaginal discharge    | 1                         | 0                                             |
| Dysuria              | 1                         | 4                                             |
| Abdominal discomfort | 1                         | 0                                             |

**Table IV.** Pathologic evaluation of vaginal lesions

| <i>Diagnosis</i>   | <i>Previous neoplasia</i> | <i>Previously benign or unrelated disease</i> |
|--------------------|---------------------------|-----------------------------------------------|
| Severe dysplasia   | 2                         | 0                                             |
| In-situ disease    | 6                         | 1                                             |
| Invasive carcinoma | 9                         | 11                                            |

follow-up after hysterectomy in this group. All but one patient had invasive carcinoma of the vagina at the time of diagnosis in the group following benign disease. All lesions were squamous cell carcinomas. One of 17 tumors in the first group and four of the 12 in the second group were reported as being poorly differentiated. The remainder of the lesions were reported as moderately or well differentiated. The clinical staging of the disease reflects the high incidence of earlier lesions in the first group, as shown in Table V. In the group with previous benign disease, eight patients or 66.6% presented with Stage II disease or greater while there were no patients with disease more advanced than Stage I in the first group.

The most common site of the tumor was in the upper third of the vagina in both groups although some tumors extended down into the lower two thirds. Only two patients had primary lesions in the middle or lower third of the vagina.

Of the patients in group 1, 16 of 17 (94%) were initially treated surgically with either a partial or total vaginectomy (Table VI). This included eight of the nine patients with invasive disease. All 10 patients with severe dysplasia or in-situ disease were treated surgically. One patient (age 79) with invasive disease was treated with radiotherapy. In this group, there were two recurrences of vaginal neoplasia with new invasive disease. One other patient was free of vaginal neoplasia but developed a transitional cell carcinoma of the bladder and subsequently died of the second cancer.

Ten of 12 of the patients (83%) in group 2 were initially treated with radiotherapy (Table VII). All 10 had invasive disease. Two patients, one with in-situ disease and one with invasive disease, were treated surgically. Four of these patients had persistent disease and



Table V. Clinical stage of disease

| Stage | Previous neoplasia | Previously benign or unrelated disease |
|-------|--------------------|----------------------------------------|
| 0     | 6                  | 1                                      |
| I     | 9                  | 3                                      |
| II    | 0                  | 4                                      |
| III   | 0                  | 3                                      |
| IVA   | 0                  | 0                                      |
| IVB   | 0                  | 1                                      |

Table VI. Treatment of patients with previous neoplasia

| Diagnosis          | No. | Treatment                           |
|--------------------|-----|-------------------------------------|
| Severe dysplasia   | 2   | Vaginectomy                         |
| In-situ disease    | 6   | Vaginectomy                         |
| Invasive carcinoma | 9   | Vaginectomy (8)<br>Radiotherapy (1) |

died as a result. Two other patients developed local recurrence and were treated with radical surgical procedures.

Survival of the patients in the two groups is markedly different (Table VIII). In group 1, 10 patients (59%) are clinically free of disease after an average of 51 months (range 24 to 72 months). One patient is alive with disease 3 years after diagnosis; two died of inter-current disease, and four were lost to follow-up. In group 2, six patients (50%) show no evidence of disease from 12 to 120 months later (average 49 months). However, five patients (42%) died of vaginal carcinoma from 2 to 8 months after diagnosis. One patient died of unknown causes.

Comment

A total of 41% of cases of squamous cell neoplasia of the vagina following hysterectomy occurred following surgery for benign or unrelated disease. Ten of 29 of these patients had no history of preceding genital tract malignancy. This figure is slightly higher than that reported by Jimerson and Merrill<sup>5</sup> (two of 11 patients) or Gallup and Morley<sup>3</sup> (five of 25 patients) in patients who had no preceding genital tract neoplasia. It would be interesting to speculate as to what etiologic factors may be present in the group without an antecedent history of genital neoplasia that might predispose them to development of malignancy after hysterectomy. This series of only 10 patients, as well as other series, are too small to allow any epidemiologic data to be significant. The average age at the time of hysterectomy in the two groups in this series is not significantly different, which contradicts the suggestion that the patients who have

Table VII. Treatment of patients with previous benign or unrelated disease

| Diagnosis       | No. | Treatment                        |
|-----------------|-----|----------------------------------|
| In-situ disease | 1   | Vaginectomy                      |
| Invasive        | 11  | Radiotherapy (10)<br>Surgery (1) |

Table VIII. Survival data of patients

|                        | Group 1:<br>Previous neoplasia | Group 2: Previous<br>unrelated disease |
|------------------------|--------------------------------|----------------------------------------|
| No evidence of disease | 10 (59%)                       | 6 (50%)                                |
| Died of disease        | 0                              | 5 (42%)                                |
| Alive with disease     | 1 (6%)                         | 0                                      |
| Died of other causes   | 2 (12%)                        | 1 (8%)                                 |
| Lost to follow-up      | 4 (23%)                        | 0                                      |

had a hysterectomy for benign disease do so at an age before they reach the high-risk age group for cervical dysplasia. However, it would seem appropriate to continue to seek out any high-risk factors that might place a patient at higher risk for the development of vaginal neoplasia following a hysterectomy for benign indications.

Certainly, the most significant predisposing factor in the development of vaginal neoplasia is previous cervical neoplasia. Seventeen of 29 patients in this series had a previous cervical lesion. The recurrence rate of carcinoma in situ of the cervix after hysterectomy is variably quoted at 0.7% to 6.6%.<sup>1</sup> This represents either persistent or multicentric disease from the initial lesion, an entirely new disease, or else a continuing susceptibility of the squamous epithelium to certain oncogenic stimulants. The fact that in this series patients presented from 1 to 22 years, with an average of 5.7 years, after the cervical lesion suggests that it is likely one of the two latter possibilities, as persistent disease would probably have been detected at an earlier stage in follow-up. Usherwood<sup>6</sup> has suggested on an empirical basis that if negative cytology exists for a period of more than 1 year after hysterectomy the vaginal lesion should then be considered a new lesion.

Several authors have attempted to make a pathologic delineation between recurrent lesions following cervical neoplasia and primary vaginal lesions. Geelhoed and associates<sup>7</sup> pointed out that primary vaginal lesions are more likely to be symptomatic and tend to show invasion at an earlier stage than recurrent cervical disease. Murad and colleagues<sup>8</sup> stated that the primary lesion is more often papillary and spreading than the

ulcerative type of secondary carcinoma. However, all of these factors may be related to the fact that patients with previous benign disease present at a more advanced clinical stage than patients with previous cervical neoplasia. At the present time, it is not possible to differentiate two distinct pathologic entities presenting as vaginal carcinoma except on a clinical basis.

A total of 21% of the patients in this series had received previous pelvic radiation. The interval between radiation and the diagnosis of vaginal neoplasia ranged from 5 to 22 years (average 14 years). Pride and Buchler,<sup>10</sup> in their series of 43 patients, showed that 20.8% of patients had received previous radiation to the upper vagina. They recommended that although the risk was low, these patients warranted particularly careful cytologic follow-up indefinitely. Murad and associates<sup>8</sup> reported that vaginal carcinomas occurring 5 or more years after treatment for cervical carcinoma had distinct histologic characteristics that would support their classification as primary vaginal lesions. It is not possible to state whether this new vaginal neoplasia is due to a particular effect of radiotherapy or a continued oncogenic susceptibility of the genital tract to a persistent stimulus.

Patients who have had a previous cervical neoplasia are usually followed up more carefully than those with previous benign or unrelated disease, as evidenced by the fact that 58% of disease in the first group versus 16% of disease in the second group was asymptomatic and detected only on routine cytologic smears. The patients in first group also all presented with in-situ or Stage I disease as opposed to only four of 12 in the second group. A greater proportion of lesions in the second group were invasive (92% versus 53%). Vaginal carcinomas are usually invasive before producing symptoms of bleeding, discharge, or dysuria, and this seriously compromises the prognosis because of the rich subepithelial lymphatics of the vagina. Therefore, patients who have had a previous hysterectomy for malignant or benign disease must be followed up routinely with cytologic smears in order to diagnose these lesions in the early stages and treat them appropriately.

In this series, in-situ disease and Stage I disease are

treated surgically with a partial or total vaginectomy in the majority of cases. Rutledge<sup>4</sup> supports this approach particularly for superficial lesions and those appearing after prior radiation. For in-situ disease or with multicentric lesions he recommends a surgical treatment method except in the presence of complicating medical diseases, advanced age, or no need for a functional vagina.<sup>11</sup> Split-thickness skin grafts may be used to restore adequate vaginal length if necessary.

If the tumor has infiltrated the submucosal tissue or if surgery is relatively contraindicated, then radiotherapy is the preferred treatment. Perez and associates<sup>12</sup> recommend intravaginal radium alone for in-situ disease or localized invasion but if there is any evidence of diffuse vaginal involvement, the parametrial and pelvic tissues should receive additional external beam therapy. Sexual disability because of contraction can be a disadvantage of radiotherapy in the younger patient, which may allow consideration of radical surgery in this group.

The small number of cases in this series precludes any significant comparisons in 5-year survival figures between the surgical and radiation modes of treatment.

In conclusion, it should be emphasized that carcinoma of the vagina may occur following hysterectomy for either benign or malignant disease. Surgery for neoplasia of the cervix must include an adequate vaginal cuff. This may be better defined by immediate preoperative colposcopic assessment to identify the margins of the lesion. Continued cytologic surveillance is mandatory in all posthysterectomy patients. Patients who have had previous pelvic radiation merit particular attention, as cytology may be misinterpreted because of the radiation-related atrophy and cellular changes.

Neoplasia of the vagina following hysterectomy for benign or unrelated disease usually presents at a more advanced stage than that following cervical neoplasia and this is likely a function of poor follow-up. In this series, in-situ and early invasive lesions are treated surgically while more advanced lesions are best treated with radiation.

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# Molar pregnancy in British Columbia: Estimated incidence and postevacuation regression patterns of the beta subunit of human chorionic gonadotropin

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A total of 171 cases of molar pregnancy were followed by serial radioimmunoassay of the beta subunit of human chorionic gonadotropin (hCG- $\beta$ ) over a 4-year period. The incidence over the study interval was 1:1,202 pregnancies in the province. In 120 women with intact uteri and measurable plasma levels of hCG- $\beta$  at the initiation of follow-up, the hormone remained detectable for up to 219 days (between 31 and 32 weeks) following evacuation, with 50% of patients remaining positive at 63 days (between 8 and 9 weeks). In five women undergoing hysterectomy, the hormone remained detectable for up to 112 days (between 15 and 16 weeks) postoperatively. In 92% of the cases, hCG- $\beta$  regressed to negative without chemotherapy. The prolonged presence of hCG in plasma after evacuation of the mole, regardless of the presence or absence of the uterus, points to persistence of molar tissue and its gradual rejection from uterine and extrauterine sites as a common phenomenon in the natural history of the uncomplicated postmolar course of this disease. (AM. J. OBSTET. GYNECOL. 139:316, 1981.)

BETWEEN 1975 AND 1978, a series of 171 women in the postmolar phase of the disease have been registered and observed in this laboratory. Preliminary data from this study have previously been reported.<sup>1, 2</sup> In this paper, the estimated incidence of this complication of pregnancy in British Columbia and the regression pattern of the concentrations of the beta subunit of human chorionic gonadotropin (hCG- $\beta$ ) during the postmolar interval of these patients are reported.

### Patients and methods

Only data from 171 patients with pathologically confirmed complete hydatid mole followed between

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**Table I.** Molar pregnancy in British Columbia, 1975 to 1978

| Year  | Total No. of cases | Total No. of pregnancies* | Incidence |
|-------|--------------------|---------------------------|-----------|
| 1975  | 25                 | 50,059                    | 1:2,002   |
| 1976  | 43                 | 50,244                    | 1:1,168   |
| 1977  | 41                 | 51,776                    | 1:1,263   |
| 1978  | 62                 | 53,604                    | 1:865     |
| Total | 171                | 205,683                   | 1:1,202   |

\*Figures from the Ministry of Health, Government of British Columbia. This includes all live births, stillbirths, and induced and spontaneous abortions.

1975 and 1978 were included (Tables I and II). During the study interval, this laboratory (in collaboration with the Cancer Control Agency of British Columbia) had undertaken the role of a trophoblastic disease registry for the province. All physicians caring for pregnant women, surgical pathologists, chemical pathologists in hospital-based and private clinical chemistry laboratories assisted in detecting new cases for registration and follow-up. In addition, all samples submitted for hCG- $\beta$  assay were checked for the diagnosis. If a sample was not accompanied by a diagnosis, a questionnaire was sent to the physician to determine if the patient had a molar pregnancy or other trophoblastic



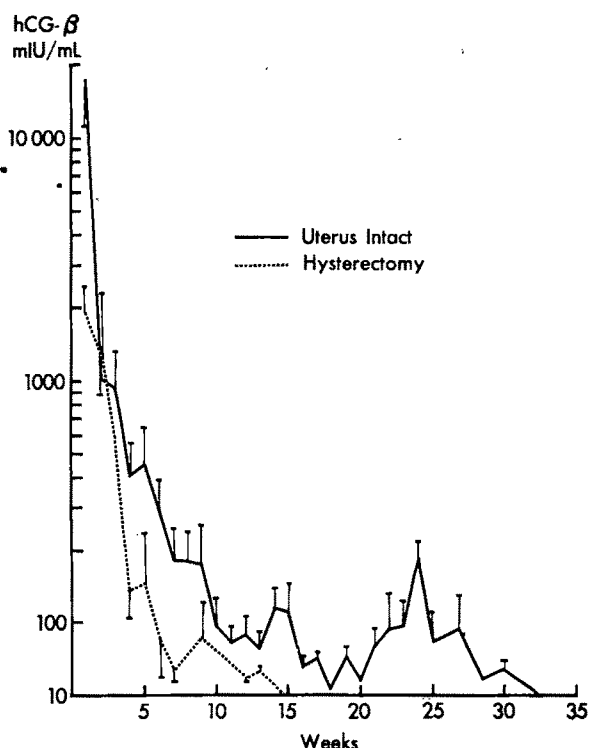


Fig. 1. Regression patterns of hCG- $\beta$  concentrations in the postmolar interval in 120 women with intact uteri and five women following hysterectomy. In four of the latter women, the mole was removed in situ, while in the remaining patient, hysterectomy was performed 4 weeks after passage of the mole.

disease. Requests for tissue pathology and operative reports were made to confirm the diagnosis. Results of hCG- $\beta$  assays and recommendations for follow-up and management were then made to the attending physician. During the study interval, this laboratory performed all the hCG- $\beta$  quantitative assays in the province. Thus, by the screening techniques noted, most cases were detected and enrolled in follow-up. Assay methodology and follow-up protocols have been described.<sup>1-3</sup> In the early part of the study,<sup>1</sup> hCG- $\beta$  was measured to a sensitivity of 10 mIU/ml and subsequently to 5 mIU/ml. In calculation of the data, values of 5 or 10 mIU/ml were assigned to samples with hCG- $\beta$  values below the level of assay sensitivity, with the latter value used in the patients studied earlier, as noted.

## Results

The incidence of molar pregnancy varied widely during the study period. Expressed as a ratio of molar to total pregnancies in the province, the average incidence over the 4-year period was 1:1,202 pregnancies (Table I).

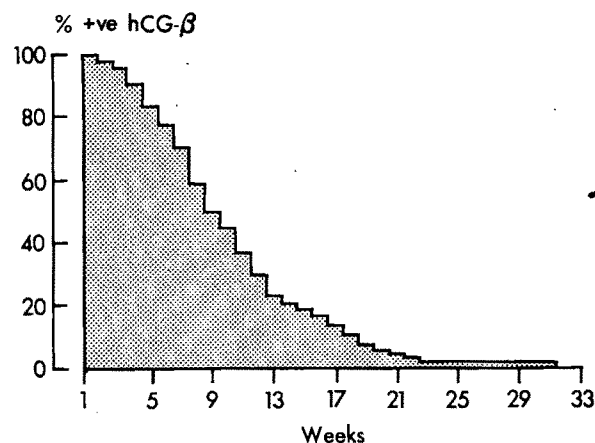


Fig. 2. hCG- $\beta$  regression after passage of molar pregnancy in 120 women with intact uteri: Percent of women with detectable plasma hCG- $\beta$  concentrations related to time in weeks during the postmolar period.

Table II. Clinical data

|                                                                                                                                                | No.  | %     | Age in years |       |
|------------------------------------------------------------------------------------------------------------------------------------------------|------|-------|--------------|-------|
|                                                                                                                                                |      |       | Mean         | Range |
| Uncomplicated molar pregnancies                                                                                                                | 156* | 91.22 | 25.1         | 13-51 |
| Molar pregnancies in patients lost to follow-up before hCG- $\beta$ became negative                                                            | 1    | 0.58  |              |       |
| Molar pregnancies followed by complications (nonmetastatic trophoblastic disease, 10; metastatic trophoblastic disease, 1; choriocarcinoma, 3) | 14†  | 8.19  | 28.5         | 19-55 |

Nonmetastatic disease: molar tissue confined to uterus; metastatic disease: molar tissue spread beyond the uterus; choriocarcinoma: no villi identified.

\*Five patients had abdominal hysterectomy, four with the mole in situ and one 4 weeks following passage of the mole. Two women (1.2%) each had two molar pregnancies.

†All received chemotherapy and three women also underwent hysterectomy.

In 156 of the 171 molar pregnancies (91.2%), hCG- $\beta$  regressed to negative without chemotherapy. In 14 (8.1%), chemotherapy was administered for postmolar complications only, while one woman was lost to follow-up before hCG- $\beta$  became negative (Table II). Of the 120 women entering the study with detectable hCG- $\beta$  plasma concentrations and intact uteri in the follow-up interval, the hormone continued to be detected for up to 219 days (between 31 and 32 weeks, Fig. 1 and Table III). Fifty percent of these patients had detectable plasma hCG- $\beta$  levels 63 days (8 to 9 weeks) after passage of the molar pregnancy (Fig. 2). In the five women who had hysterectomy performed, hCG- $\beta$  remained detectable in plasma for up to 112

**Table III.** Comparison of data on regression patterns for hCG- $\beta$  plasma concentrations in molar pregnancy, depending on the presence or absence of the uterus in the postmolar interval

|                | No. of molar pregnancies | Age in years (mean $\pm$ SEM) | No. of days to negative hCG- $\beta$ plasma level |        |
|----------------|--------------------------|-------------------------------|---------------------------------------------------|--------|
|                |                          |                               | Mean $\pm$ SEM                                    | Range  |
| Uterus intact  | 120                      | 24.79 $\pm$ 2.96              | 73.63 $\pm$ 3.81                                  | 11-219 |
| Uterus removed | 5*                       | 42.40 $\pm$ 3.78              | 75.80 $\pm$ 11.46                                 | 49-112 |

\*In four women, the mole was removed in situ, while in the remaining women, hysterectomy was performed 4 weeks after passage of the mole.

days (between 15 and 16 weeks) postoperatively (Fig. 1). The average time for hCG- $\beta$  to reach undetectable levels in women with intact uteri was 73.6 days and in those who underwent hysterectomy it was 75.8 days (Table III).

#### Comment

The 171 cases included do not represent all molar pregnancies in British Columbia since this condition is not required to be reported here.

Over the study period, the incidence varied widely, averaging 1:1,202 pregnancies in the province (Table I). These figures, for a Canadian province, suggest that molar pregnancy was at least as common here as elsewhere; the incidence was 1:1,200 pregnancies in North Carolina,<sup>4</sup> and a general incidence of 1:2,000 to 1:2,500 pregnancies was quoted for the entire United States with a tenfold or greater incidence in Mexico and Asia.<sup>5</sup> In our series, repeat molar pregnancy occurred in 1.2% of the women, a figure comparable to 1.4% reported from the Southeastern Regional Trophoblastic Disease Center.<sup>4</sup> Fourteen women (8.2%) required chemotherapy (Table II), while in all other patients (except for the one lost to follow-up), the hCG- $\beta$  levels regressed spontaneously without chemotherapy.

In 120 patients who entered the study with detectable plasma concentrations of hCG- $\beta$ , the hormone remained detectable for up to 219 days (between 31 and 32 weeks). In contrast, in five women who underwent hysterectomy with the mole in situ or soon after passage, the hormone remained detectable for up to 112 days (between 15 and 16 weeks) postoperatively, about half the time in comparison to patients with intact uteri (Table III). However, the average time for plasma hCG- $\beta$  to regress to negative was similar in women with intact uteri and those who underwent hysterectomy. This time interval of 73 to 76 days (uterus intact and hysterectomy groups, respectively) agrees

with the average time of 79 days reported during the early part of the study.<sup>1</sup> Mean hCG- $\beta$ -positive intervals of 90 and 55 days were reported in the postmolar period with intact uteri and when the uterus was removed with the mole in situ, respectively.<sup>6</sup> With intact uteri and induced abortion of normal pregnancy, this interval was 37 and 27 days in the first and second trimester, respectively. Hysterectomy in the second trimester with a normal pregnancy in situ resulted in mean hCG- $\beta$ -positive intervals of 39 days with a reduction to 12 days when the hysterectomy was initiated with ligation of the uterine and ovarian vessels.<sup>7</sup> After normal delivery at term, hCG- $\beta$  disappears from maternal plasma between 11 and 16 days.<sup>8</sup> These observations are explicable on the basis of the high levels of hCG during the first trimester of pregnancy, the relatively long plasma half life of the hormone,<sup>9</sup> and the morphologic observations demonstrating deportation of trophoblast to extrauterine sites during normal and molar pregnancy.<sup>5</sup>

This series of molar pregnancies monitored by serial hCG- $\beta$  radioimmunoassay in a laboratory given the responsibility of detection, registration, and follow-up of women with molar pregnancy resulted in good patient compliance and low rates of chemotherapy, which was administered only for complications in the postmolar follow-up interval. Finally, the prolonged presence of hCG- $\beta$  in plasma during the postmolar phase, regardless of the presence or absence of the uterus, points to the persistence of molar trophoblast and its gradual rejection from uterine and extrauterine sites as a common phenomenon in the natural history of the uncomplicated postmolar course of this disease.

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# Puerperal febrile complications and cervical flora following elective manual exploration of the uterus

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Two hundred patients who underwent vaginal delivery were randomly divided into two groups on arrival to the case room in labor. After delivery was completed the uteri of 100 patients were manually explored; the remainder of the patients served as a control group. Aerobic and anaerobic cultures of the endocervix were taken from 50 patients in each group, during labor and on the fourth postpartum day. There was no difference in the number of puerperal febrile complications between the groups. Endocervical cultures showed a marked increase in the incidence of *Escherichia coli* and anaerobic bacteria after delivery. This increase occurred in both groups in similar proportions and was not affected by manual exploration. (AM. J. OBSTET. GYNECOL. 139:320, 1981.)

THE SAFETY AND CONVENIENCE of routine manual exploration of the uterus following delivery is still a controversial issue among obstetricians. Numerous favorable reports exist,<sup>1-5</sup> but most of these studies were retrospective and failed to provide a definite answer because of deficient randomization, lack of statistical analysis of results, prophylactic use of antibiotics, etc. We were able to find only one prospective, controlled study confirming the absence of complications.<sup>6</sup> On the other hand, vaginal and cervical flora have been studied at different stages of pregnancy and in the puerperium,<sup>7-10</sup> but it is not known how cervical flora is influenced by manual exploration of the uterine cavity. The purpose of this prospective study was to find out whether there was an increased incidence of endometritis, urinary tract infection, or other febrile complications following elective exploration of the uterus and the effect of this procedure on the cervical flora during the early puerperium.

## Material and methods

The study population included mostly private patients, some public clinic patients, and some patients

referred from other centers. All patients were delivered at the Ottawa General Hospital between February 13 and June 27, 1979, by the resident on call and the patient's private physician. It was decided to include 200 patients undergoing vaginal delivery, either spontaneous or instrument-assisted, and randomize them in two groups according to their chart numbers. On arrival to the case room, patients were assigned to the "exploration" group if they had an odd chart number and to the "no-exploration" group if they had an even chart number. After delivery was completed and the placenta was spontaneously delivered, patients in the "exploration" group all underwent manual exploration of the uterus according to standard technique: Without a change in drapes or gloves, the anterior, posterior, and cornual regions of the uterus were systematically explored for placental remnants. Patients in the "no-exploration" group did not undergo elective exploration; however, if a medical indication such as postpartum bleeding or retained placenta made manual exploration imperative, this was done and the patient was excluded from the study. Patients who required delivery by cesarean section were also excluded from the study.

**Febrile complications.** Postpartum fever was defined as a temperature elevation of 37.5° C on any two occasions after delivery separated by at least 6 hours, exclusive of the first 24 hours. Endometritis was defined as postpartum fever associated with uterine tenderness. The diagnosis of urinary tract infection was made when there was a culture of more than

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100,000 colonies per milliliter of urine, in the presence of typical symptoms. Patients with positive urine culture but no symptoms were considered to have asymptomatic bacteriuria and were not listed as having febrile complications.

**Bacteriology.** Fifty patients from each group were cultured during labor before the first pelvic examination and an equal number of patients were cultured on the fourth postpartum day, which made results easily comparable. These patients were chosen at random. Many patients were cultured on both occasions, but some of them were cultured only once, either during labor or in the puerperium. In order to avoid contamination from the vagina, the cervix was clearly visualized with an unlubricated speculum and two sterile swabs were taken from the endocervix, one for aerobes and one for anaerobes. Swabs for aerobes were transported to the laboratory in Amies charcoal transport medium and were used for inoculation of the culture media and Gram stain. Swabs for anaerobes were transported in an anaerobic collector (Baltimore Biological Laboratory) and also used for inoculation and Gram stain. Aerobic incubation was done in: (1) 5% sheep blood agar in 5% carbon dioxide at 35° C overnight, (2) modified Thayer-Martin medium in 5% carbon dioxide at 35° C during 5 days with daily checking; and (3) MacConkey's agar overnight. Anaerobic incubation was done in: (1) CBV agar and PAM agar in a Gas-Pak anaerobic jar for 48 hours and (2) thioglycolate broth at 35° C for 5 days, with daily checking for turbidity. Anaerobic identification was done by using the API 20 A system.

The z test for proportions was used to detect a statistically significant difference at the 5% level between the proportions of positive cultures in the two groups (exploration and no-exploration).

Results

A total of 100 patients in each group were included in the study. Table I shows the obstetric characteristics of the groups. The number of primiparous and multiparous patients in both groups was similar. Most of the patients had either spontaneous or low-forceps delivery under epidural anesthesia. There was a greater incidence of prolonged labor (more than 18 hours) in the no-exploration group, but it should be noted that none of these patients was febrile during the postpartum period.

Table II shows the incidence and diagnosis of patients with febrile complications. Patients with fever of unexplained origin were all asymptomatic, had a negative physical examination except for variable breast engorgement, and became afebrile without specific treat-

Table I. Obstetric characteristics

| Characteristic                 | No-exploration group<br>(100 patients) | Exploration group<br>(100 patients) |
|--------------------------------|----------------------------------------|-------------------------------------|
| Parity:                        |                                        |                                     |
| Primiparous                    | 50                                     | 45                                  |
| Multiparous                    | 50                                     | 55                                  |
| Type of delivery:              |                                        |                                     |
| Spontaneous                    | 61                                     | 66                                  |
| Low-forceps                    | 24                                     | 23                                  |
| Mid-forceps                    | 9                                      | 6                                   |
| Vacuum extractor               | 6                                      | 5                                   |
| Anesthesia:                    |                                        |                                     |
| Epidural                       | 58                                     | 61                                  |
| Local                          | 32                                     | 28                                  |
| General                        | 0                                      | 2                                   |
| None                           | 10                                     | 9                                   |
| Labor:                         |                                        |                                     |
| Prolonged rupture of membranes | 2                                      | 2                                   |
| Prolonged labor (+18 hours)    | 9                                      | 0                                   |

Table II. Number of patients with febrile complications

| Complication                 | No-exploration group<br>(100 patients) | Exploration group<br>(100 patients) |
|------------------------------|----------------------------------------|-------------------------------------|
| Endometritis                 | 1                                      | 1                                   |
| Urinary tract infection      | 1                                      | 1                                   |
| Episiotomy infection         | 1                                      | 0                                   |
| Superficial thrombophlebitis | 0                                      | 1                                   |
| Unexplained                  | 2                                      | 2                                   |
| Total                        | 5                                      | 5                                   |

ment. There were five patients with asymptomatic bacteriuria in each group who are not listed in Table II.

Table III lists the number of positive endocervical cultures for the aerobic organisms during labor and on the fourth postpartum day. It can be seen that the nonpathogenic organisms were most frequently found during labor among the noexploration and the exploration patients, but their incidence decreased after delivery. The opposite was true for *Escherichia coli*, which was not commonly found during labor but had the highest incidence during the postpartum period. There was no statistical difference between the proportions of positive cultures in either group for any of the organisms ( $p > 0.05$ ).

Table IV lists the number of positive endocervical cultures for anaerobic organisms during labor and on the fourth postpartum day. Most of the anaerobes showed a higher incidence in the postpartum period. No statistical difference was found between the proportions of positive cultures in the no-exploration and the exploration groups ( $p > 0.05$ ).

Table III. Number of positive endocervical cultures for aerobes during labor and on the fourth postpartum day

| Organism                           | No-exploration group<br>(50 patients) |             | Exploration group<br>(50 patients) |             |
|------------------------------------|---------------------------------------|-------------|------------------------------------|-------------|
|                                    | Labor                                 | Post partum | Labor                              | Post partum |
| Probable nonpathogens:             |                                       |             |                                    |             |
| Lactobacillus                      | 22                                    | 7           | 18                                 | 3           |
| Staphylococcus epidermidis         | 20                                    | 8           | 17                                 | 5           |
| Diphtheroids                       | 0                                     | 2           | 0                                  | 0           |
| Gram-positive cocci:               |                                       |             |                                    |             |
| α-Hemolytic streptococcus          | 0                                     | 1           | 0                                  | 3           |
| β-Hemolytic streptococcus, group B | 2                                     | 6           | 1                                  | 2           |
| Nonhemolytic streptococcus         | 7                                     | 11          | 7                                  | 9           |
| Enterococcus                       | 0                                     | 0           | 1                                  | 3           |
| Streptococcus bovis                | 0                                     | 2           | 0                                  | 0           |
| Staphylococcus aureus              | 0                                     | 1           | 1                                  | 0           |
| Gram-negative enteric bacilli:     |                                       |             |                                    |             |
| Escherichia coli                   | 5                                     | 21          | 3                                  | 19          |
| Klebsiella pneumoniae              | 0                                     | 2           | 0                                  | 3           |
| Enterobacter                       | 0                                     | 0           | 0                                  | 2           |
| Citrobacter                        | 0                                     | 0           | 0                                  | 1           |
| Proteus sp.                        | 0                                     | 1           | 0                                  | 1           |
| Others:                            |                                       |             |                                    |             |
| Haemophilus vaginalis              | 0                                     | 1           | 0                                  | 1           |
| Candida albicans                   | 9                                     | 2           | 6                                  | 1           |
| Total                              | 65                                    | 65          | 54                                 | 53          |

Table IV. Number of positive endocervical cultures for anaerobes during labor and on the fourth postpartum day

| Organism                           | No-exploration group<br>(50 patients) |             | Exploration group<br>(50 patients) |             |
|------------------------------------|---------------------------------------|-------------|------------------------------------|-------------|
|                                    | Labor                                 | Post partum | Labor                              | Post partum |
| Gram-positive cocci:               |                                       |             |                                    |             |
| Peptococcus                        | 3                                     | 7           | 6                                  | 9           |
| Peptostreptococcus                 | 4                                     | 9           | 6                                  | 7           |
| Gram-positive bacilli:             |                                       |             |                                    |             |
| Clostridium perfringens            | 0                                     | 0           | 0                                  | 1           |
| Unidentified gram-positive bacilli | 3                                     | 1           | 4                                  | 2           |
| Gram-negative bacilli:             |                                       |             |                                    |             |
| Bacteroides fragilis               | 0                                     | 0           | 0                                  | 1           |
| Bacteroides melaninogenicus        | 0                                     | 1           | 0                                  | 1           |
| Bacteroides sp.                    | 0                                     | 7           | 1                                  | 7           |
| Total                              | 10                                    | 25          | 17                                 | 28          |

Comment

This prospective study confirms the safety of routine manual exploration of the uterus and, therefore, is in agreement with most previous reports.<sup>1-6</sup> The number of patients with febrile complications was exactly the same in both groups, as was the number of patients with asymptomatic bacteriuria. The incidence of febrile complications of 5% in both groups may seem somewhat high for our study population, but this is probably due to our definition of postpartum fever. The classic definition of febrile morbidity, given by the Joint Committee on Maternal Welfare, which is "a temperature of 38° C on any two of the first 10 days postpartum

exclusive of the first 24 hours," may not be appropriate in a setting where patients are aggressively treated with antibiotics as soon as a febrile complication is diagnosed. Since only one patient in each group of our study had a temperature of more than 38° C on two different days post partum, our incidence of febrile morbidity was only 1% according to the classic definition.

Our bacteriologic findings were also in agreement with those of previous reports,<sup>7-10</sup> but we believe this is the first time that the impact of manual exploration on the aerobic and anaerobic cervical flora has been assessed. The total number of positive cultures for



aerobes remained almost unchanged after delivery in both groups, but there was an increase in the incidence of gram-positive cocci, and especially of *Escherichia coli*, over *Lactobacillus*, *Staphylococcus epidermidis*, and *Candida albicans*. The total number of positive cultures for anaerobes increased after delivery, with a remarkable rise in the number of bacteroides. These changes were not influenced by manual exploration of the uterus, as they occurred in both groups without a statistically significant difference. The higher incidence of *Escherichia coli* and anaerobic bacteria during the early puerperium suggests cervical colonization from the bowel at the time of delivery. The exact mechanism is not entirely understood, but contamination with feces is most likely an important factor.

Some criticism can be applied to this study. The number of patients who were cultured was small and there might have been a certain selection since we were

not able to culture some patients who presented in very advanced labor and some others who were discharged before the fourth postpartum day. However, these shortcomings applied equally to both groups and should have been offset by randomization. Only two patients who were originally assigned to the no-exploration group had to be explored for medical indications and were withdrawn from the study. Neither of them was febrile post partum.

While the advantages of routine manual exploration are not clearly established, our findings suggest that the procedure neither increases febrile morbidity nor significantly alters the cervical flora during the early puerperium.

We thank Dr. Rama Nair for doing the statistical analysis.

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# Effects of synthetic glucocorticoid administration on human fetal breathing movements at 34 to 35 weeks' gestational age

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Fetal breathing movements were measured in six women who had received exogenous glucocorticoids throughout pregnancy and in whom the endogenous circadian rhythm in cortisol was absent or had been suppressed. Studies were performed for 24-hour observation periods while women received prednisone and then one week later on the same women after equivalent doses of dexamethasone. Fetal breathing movements occurred  $29.9\% \pm 1.9\%$  of the time in the prednisone-treated group and this was not changed in the same fetuses after dexamethasone treatment. There was a significant increase in the percentage of time spent making breathing movements during the second and third hours following breakfast, lunch, and dinner ( $P < 0.001$ ). A fourth peak in fetal breathing activity occurred in the late evening and seemed to be related to the time of the evening glucocorticoid medication. These data were compatible with the hypothesis that maternal glucocorticoid concentrations may influence patterns of fetal breathing movements at 34 to 35 weeks' gestational age. (AM. J. OBSTET. GYNECOL. 139:324, 1981.)

WE HAVE REPORTED that at 34 to 35 weeks' gestational age the incidence of fetal breathing movements was increased during the second and third hours following maternal meals and that a prolonged significant increase in fetal breathing movements occurred overnight while mothers were asleep.<sup>1</sup> The increase in fetal breathing activity during the day, following maternal

meals, was reproduced by giving fasted women glucose and no similar increase occurred during the day when women were not fed or given glucose.<sup>2</sup> The prolonged significant increase in fetal breathing movements measured overnight occurred during a time when maternal plasma glucose concentrations were stable or falling. It was hypothesized that the overnight increase in fetal breathing movements might be part of a circadian rhythm in fetal activity.<sup>1</sup>

At 34 to 35 weeks, we demonstrated that a significant circadian rhythm existed in the concentrations of cortisol and estriol in maternal venous plasma and that there was an inverse relationship between these steroids.<sup>3, 4</sup> These data were consistent with the hypothesis that maternal glucocorticoids may influence fetal hypothalamic-pituitary-adrenal function. It was interesting that the overnight increase in fetal breathing movements at 34 to 35 weeks occurred at about the same time as the increase in maternal glucocorticoids. In order to test the hypothesis that maternal glucocorticoids may influence fetal breathing activity, we examined changes in fetal breathing movements in a

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Table I. Glucocorticoid indications and daily dosage

| Patient No. | Indications for glucocorticoid                                              | Prednisone dosage (mg) | Dexamethasone dosage (mg) |
|-------------|-----------------------------------------------------------------------------|------------------------|---------------------------|
| 1           | Lupus erythematosus, in remission for 4 years                               | 15                     | 2.0                       |
| 2           | Ulcerative colitis, in remission for 4 years                                | 10                     | 1.5                       |
| 3           | Chronic asthma, in remission for 1 year                                     | 20                     | 2.0                       |
| 4           | Adrenal insufficiency since age 14                                          | 20                     | 2.0                       |
| 5           | Pituitary insufficiency (pituitary adenectomy 3 yr before)                  | 10                     | 1.5                       |
| 6           | Adrenal genital syndrome, hirsutism, acne, infertility syndrome for 4 years | 15                     | 2.0                       |

Table II. Clinical data of fetal outcome

| Patient No. | Gestation at birth (Wks) | Sex | Birth weight (gm) | Placental weight (gm) | Apgar score at 5 min | Umbilical arterial cord blood |                          |      |              |                      |             |
|-------------|--------------------------|-----|-------------------|-----------------------|----------------------|-------------------------------|--------------------------|------|--------------|----------------------|-------------|
|             |                          |     |                   |                       |                      | PO <sub>2</sub> (mm Hg)       | PCO <sub>2</sub> (mm Hg) | pH   | Bicar-bonate | Total carbon dioxide | Base excess |
| 1           | 41                       | F   | 3,350             | 420                   | 9                    | 18                            | 30                       | 7.29 | 14.2         | 15                   | -11         |
| 2           | 38                       | F   | 2,830             | —                     | 9                    | —                             | —                        | —    | —            | —                    | —           |
| 3           | 38                       | F   | 2,920             | 610                   | 10                   | 45*                           | 37                       | 7.37 | 21.2         | 22.3                 | -3          |
| 4           | 40                       | F   | 2,460             | 550                   | 9                    | —                             | —                        | —    | —            | —                    | —           |
| 5           | 39                       | F   | 3,540             | —                     | 9                    | 25                            | 48                       | 7.23 | 19.5         | 21                   | -8          |
| 6           | 42                       | F   | 3,040             | 570                   | 9                    | 23                            | 50                       | 7.22 | 19.6         | 21.1                 | -8          |
| Mean        |                          |     | 3,023             | 538                   | 9.3                  | 27.8                          | 41.3                     | 7.28 | 18.6         | 19.9                 | -7.5        |
| SD          |                          |     | 385               | 82                    | 0.5                  | 11.9                          | 9.4                      | 0.07 | 3.1          | 3.3                  | 3.3         |
| SE          |                          |     | 157               | 41                    | 0.2                  | 5.9                           | 4.7                      | 0.04 | 1.5          | 1.6                  | 1.7         |

\*Umbilical vein.

group of women treated with synthetic glucocorticoids throughout pregnancy and in whom the endogenous circadian rhythm in cortisol was absent or had been suppressed.

Patients

Informed consent was obtained from six healthy pregnant women at 34 weeks' gestational age who had received exogenous glucocorticoids throughout pregnancy for medical indications. The details of dosage and medications are noted in Table I. Fetal outcome confirmed the good health of patients studied (Table II). The average weight of fetuses at delivery was 3,023 grams and the average Apgar score at 5 minutes was 9.3. There was no Apgar score at 5 minutes less than 9 and no umbilical arterial pH less than 7.22. None of the fetuses developed fetal distress during labor.

Experimental design

Each of the six patients was studied for two 24-hour intervals. At 34 weeks' gestational age the six patients who were receiving prednisone were studied continuously from 0800 to 0800 hours the following day. After the first 24-hour study, patients were sent home; during the following week prednisone was discontinued and they were given dexamethasone in an equivalent dosage (Table I) for 3 days. A second study

Table III. Mean carbohydrate, protein, fat, and calorie intake of patients at breakfast, lunch, and dinner

|                   | Breakfast (0800 hours) | Lunch (1200 hours) | Dinner (1700 hours) |
|-------------------|------------------------|--------------------|---------------------|
| Carbohydrate (gm) | 61.2                   | 58.2               | 57.6                |
| Protein (gm)      | 22.8                   | 24.9               | 20.1                |
| Fat (gm)          | 18.0                   | 22.6               | 19.9                |
| Total (kcal)      | 497.8                  | 554.2              | 487.1               |

was performed on the fourth day of dexamethasone therapy, during which time the patients were restudied continuously for 24 hours, from 0800 to 0800 hours the following day. The dosage of dexamethasone was based on the fact that the glucocorticoid and anti-inflammatory potency ratio of dexamethasone to prednisone is 7.5:1.<sup>5</sup>

The remaining part of the experimental design has been described elsewhere.<sup>1</sup> Briefly, patients rested in bed in a quiet room exposed to normal daylight and darkness. The women were given 800 kcal meals at 0800, 1200, and 1700 hours. The actual amounts of carbohydrate, protein, and fat ingested were recorded and were not significantly different in the two studies (Table III). No other food was given to patients. Smoking was not permitted by patients or observers.

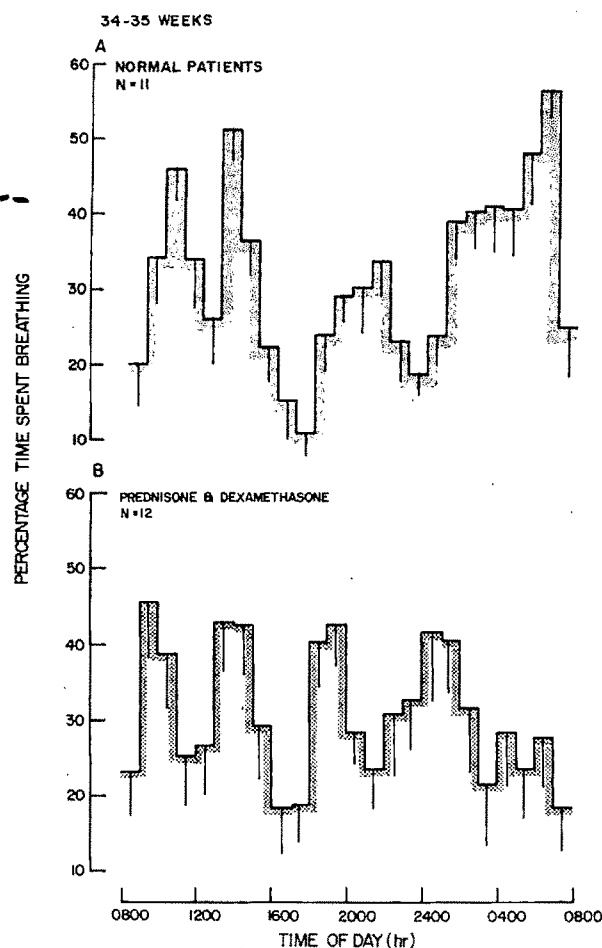


Fig. 1. A: The percentage of time spent breathing ( $\pm$ SEM) in fetuses of 11 normal patients not receiving glucocorticoids. (Redrawn from Patrick, J., Natale, R., and Richardson, B.: *AM. J. OBSTET. GYNECOL.* 132:507, 1978.) B: The percentage of time spent breathing per hour by the fetuses in this study whose mothers were given synthetic glucocorticoids. In both normal and glucocorticoid-treated patients fetal breathing movements increased during the second and third hours following breakfast, lunch, and dinner. However, in glucocorticoid-treated patients there was a fourth peak in breathing activity at 1200 to 0200 hours, and no significant peak in fetal breathing activity occurred overnight, between 0200 and 0700 hours.

Every 2 hours during the 24-hour observation periods, 5 ml of free-flowing maternal peripheral venous blood was sampled and blood for glucose determinations was analyzed at the end of each study as a batch by means of a glucose-oxidase oxygen ratemeter.\* Measurements of plasma cortisol, progesterone, estriol, estradiol, and estrone were also performed and were reported elsewhere.<sup>6</sup>

\*Beckman Instruments, Schiller Park, Illinois.

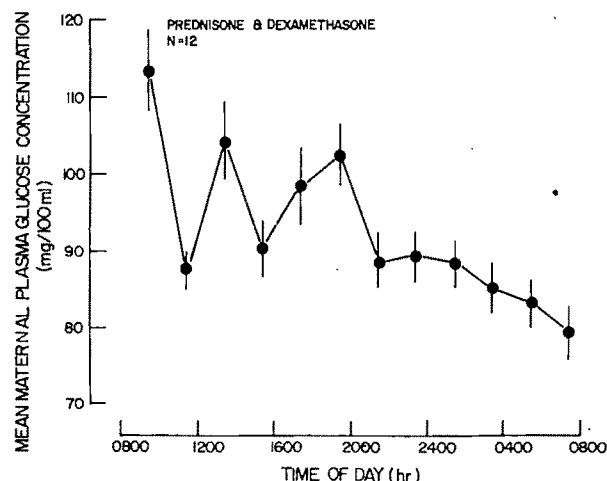


Fig. 2. Maternal plasma glucose concentrations were measured every 2 hours during the 24-hour observation period. Peak glucose concentrations occurred at 0930, 1330, and 1930 hours. Glucose concentrations fell slowly from 1930 hours and were lowest at 0730 hours in the morning.

#### Analysis of fetal breathing movements

Observations of fetuses were made with an ADR real-time ultrasonic scanner\* by a method previously described and validated.<sup>1, 7, 8</sup> The transducer was operated at a frequency of 3.5 MHz with an average intensity of 0.045 mW/sq cm.

Results presented represent grouped means and standard error of the means for each hour of the two recording periods. Significance was determined by use of a *t* test for small samples, with variance not assumed to be equal, as described by Bailey.<sup>9</sup>

#### Results

**Fetal breathing movements in prednisone-treated patients.** The six fetuses made breathing movements  $29.9\% \pm 1.9\%$  of the time (range 15.0% to 45.5%). The percentage of time spent breathing in any 1 hour of the 144 hours of observation time varied from 0 to 88.3. During episodes of breathing, the mean rate of fetal breathing movements was  $46.2 \pm 1.0$  breaths per minute. Absence of fetal breathing activity was observed for up to 73.7 minutes during 144 hours of observation time. There were 13 1-hour periods during which time there was less than 1 minute of fetal breathing observed and three 2-hour periods during which time less than 1 minute of fetal breathing was observed. The mean hourly percentage of time spent making gross fetal body movements was  $11.0\% \pm 0.7\%$  in this group of patients.

\*Model 2130, ADR Ultrasound, Tempe, Arizona.



**Fetal breathing movements in the dexamethasone-treated patients.** The six fetuses made breathing movements  $31.9\% \pm 2.0\%$  of the time (range 17.2% to 47.7%). The percentage of time spent breathing in any 1 hour of the 144 hours of observation time varied from 0 to 80.2. During episodes of breathing, the mean rate of fetal breathing movements was  $48.6 \pm 1.0$  breaths per minute. Absence of fetal breathing activity was observed for up to 63.8 minutes. During the 144 hours of observation time there were 13 1-hour periods during which time less than 1 minute of fetal breathing activity was observed and five 2-hour periods during which time less than 1 minute of fetal breathing activity was observed.

**Patterns of fetal breathing movements and glucose concentrations in prednisone- and dexamethasone-treated patients.** Patterns in fetal breathing movements and maternal glucose concentrations were not significantly different in the two treatment groups and data were presented as grouped means for the purposes of this paper. Data from a group of normal (untreated) women at 34 to 35 weeks' gestation were plotted for comparison.<sup>1</sup>

The percentage of time spent breathing by fetuses was related to ingestion of food by mothers (Fig. 1). There was a significant increase in fetal breathing activity during the second and third hours following maternal meals. From 0700 to 0900 hours fetuses breathed  $20.6\% \pm 4.0\%$  of the time and this increased significantly to  $42.1\% \pm 5.0\%$  of the time from 0900 to 1100 hours ( $P < 0.005$ ). Fetuses made breathing movements 25.8% of the time from 1100 to 1300 hours and breathing movements increased significantly to  $42.8\% \pm 4.4\%$  of the time from 1300 to 1500 hours ( $P < 0.025$ ). At 1600 to 1800 hours fetuses made breathing movements  $18.6\% \pm 3.8\%$  of the time and this increased significantly to  $41.4\% \pm 3.8\%$  of the time at 1800 to 2000 hours ( $P < 0.001$ ). Therefore, during the second and third hours following breakfast, lunch, and dinner in fetuses whose mothers received prednisone or dexamethasone, there was a significant increase in the percentage of time spent breathing.

Maternal peripheral plasma glucose concentrations increased significantly after each meal (Fig. 2). A peak of  $113.3 \pm 5.1$  mg/100 ml was measured at 0930 hours following breakfast; a peak of  $104.3 \pm 4.9$  mg/100 ml was measured at 1300 hours following lunch, and a peak of  $102.8 \pm 3.9$  mg/100 ml was measured at 1930 hours following dinner. Glucose concentrations declined slowly from 1930 hours and reached the lowest concentration of  $75.8 \pm 2.1$  mg/100 ml at 0730 hours before breakfast.

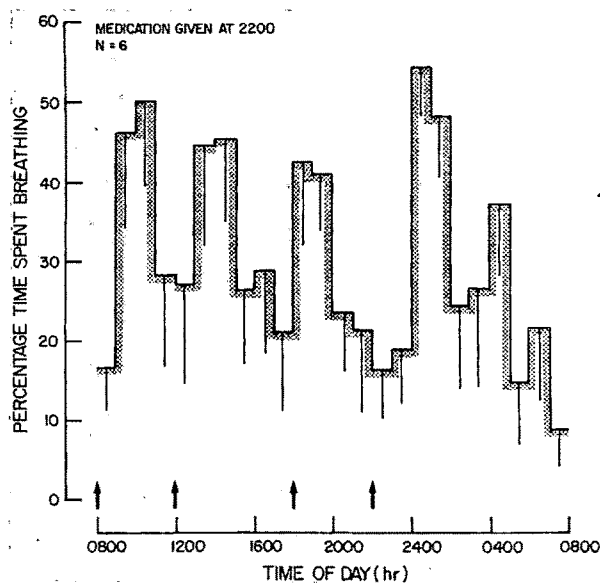


**Fig. 3.** The percentage of time spent breathing by six fetuses whose mothers received glucocorticoid at 2200 hours was plotted each hour of the day. During the day, it was impossible to separate effects of glucocorticoid from effects of meals but a significant peak in fetal breathing activity between 2400 and 0200 hours was not related to meals or maternal glucose and may have been related to glucocorticoid medication at 2200 hours.

A fourth peak in fetal breathing activity occurred between 2400 and 0200 hours, during which time fetuses made breathing movements  $41.0\% \pm 5.6\%$  of the time, which was significantly greater than that during the 4 hours following the peak (0200 to 0600 hours) when the fetuses made breathing movements  $26.2\% \pm 3.7\%$  of the time ( $P < 0.05$ ). Fetuses made breathing movements  $28.8\% \pm 3.0\%$  of the time from 2000 to 2400 hours, which was not significantly less than the peak between 2400 and 0200 hours ( $P < 0.1$ ).

The peak at 2400 to 0200 hours was most pronounced in fetuses whose mothers received either prednisone or dexamethasone at 2200 hours (prednisone,  $n = 2$ ; dexamethasone,  $n = 4$ ) (Fig. 3). In these fetuses, the percentage of time spent breathing between 2400 and 0200 hours was  $51.3\% \pm 4.6\%$ . This was significantly greater than during the 4 hours before (2000 to 2400 hours), when fetuses made breathing movements  $20.0\% \pm 3.6\%$  of the time ( $P < 0.001$ ), and was greater than during the 4 hours following (0200 to 0600 hours), when fetuses made breathing movements  $25.7\% \pm 4.9\%$  of the time ( $P < 0.001$ ).

Following the peak in fetal breathing activity between 2400 and 0200 hours, there was no significant increase in fetal breathing movements overnight while mothers were asleep. Fetuses made breathing move-

ments  $24.9\% \pm 4.3\%$  of the time between 0200 and 0700 hours and  $24.5\% \pm 5.3\%$  of the time between 0400 and 0700 hours.

### Comment

In this study of six women who received synthetic glucocorticoids for medical indications throughout pregnancy, there were four peaks in fetal breathing movements over 24-hour observation periods. The patterns in fetal breathing activity were similar whether mothers received prednisone or dexamethasone. There was a significant increase in fetal breathing movements during the second and third hours after each meal. This observation was similar to that in a previous report of patterns of fetal breathing activity in a group of normal women not receiving glucocorticoids at 34 to 35 weeks' gestational age.<sup>1</sup> It has been demonstrated that peaks in breathing activity following maternal meals can be reproduced by giving women glucose after fasting.<sup>2</sup> Significant peaks in fetal breathing activity do not occur during the day when women are not fed or given glucose. Therefore, at 34 to 35 weeks, exogenous glucocorticoids did not seem to change the pattern in fetal breathing activity, which is related to maternal meals during the day.

A peak in fetal breathing activity was measured at 2400 to 0200 hours and was most pronounced in women who received glucocorticoid at 2200 hours. This evening peak was not related to meals and maternal plasma glucose concentrations were stable or falling. It is possible that the increase in fetal breathing activity between 2400 and 0200 hours was related to the evening glucocorticoid administration. No similar

peak in fetal breathing activity was measured in the late evening in similar studies of normal women at 34 to 35 weeks who did not receive glucocorticoids.<sup>1</sup> During the rest of the day it was impossible to separate effects of meals from effects of glucocorticoids because women received their medication with meals.

In healthy fetuses whose mothers did not receive any exogenous glucocorticoid or other medication, at 30 to 39 weeks' gestation, it was reported that a prolonged significant increase occurred in fetal breathing activity between 0400 and 0700 hours while mothers were asleep.<sup>1, 10</sup> In these same women maternal cortisol concentrations were measured and rose by 131% on average from a low at 0030 to 0230 hrs to a peak at 0730 to 0830 hours.<sup>4</sup> In the present study, no similar peak in fetal breathing movements occurred overnight and measurements of maternal cortisol concentrations demonstrated small changes overnight.<sup>6</sup> Furthermore, in the present study there was an increase in fetal breathing movements at 2400 to 0200 hours, which was most prominent in women treated with glucocorticoid at 2200 hours. It is tempting to conclude from these observations that fetal breathing movements are increased by an increase in maternal glucocorticoid, whether synthetic or natural. It is possible that glucocorticoids may cross the placenta<sup>11</sup> and act to increase fetal breathing movements directly or indirectly, perhaps by increasing fetal glucose concentrations.

We wish to thank Dr. P. Harding for his interest in this work and Mrs. T. Clarke, Miss M. Harding, Miss C. Hurst, Ms. T. Vandenberg, and Mr. D. Miller for their excellent technical assistance.

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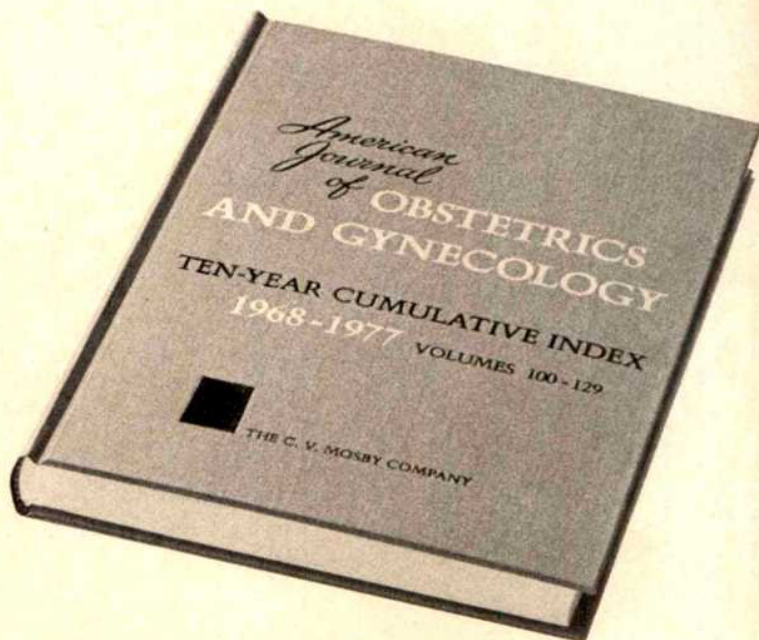
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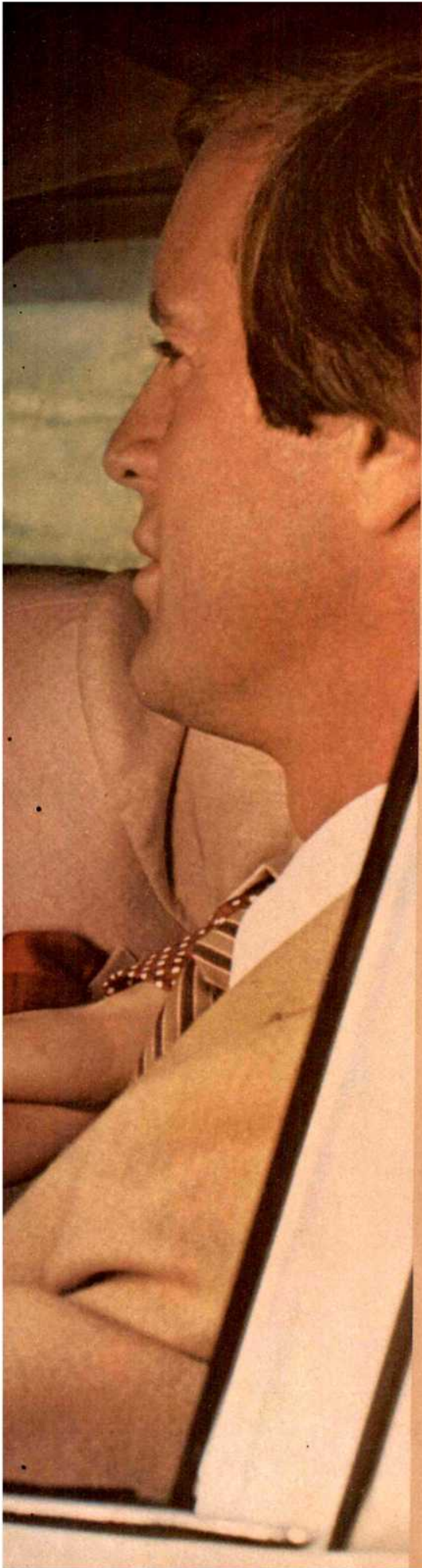


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### DESCRIPTION

Loestrin 1.5/30 Products are progestogen-estrogen combinations.

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Loestrin 1.5/30 Products are indicated for the prevention of pregnancy in women who elect to use oral contraceptives.

In clinical trials with Loestrin 1.5/30 involving 17,139 therapy cycles, there was a pregnancy rate of 0.49 per 100 woman-years.

**Dose-related risk of thromboembolism from oral contraceptives:** Studies have shown a positive association between the dose of estrogens in oral contraceptives and the risk of thromboembolism. It is prudent and in keeping with good principles of therapeutics to minimize exposure to estrogen. The oral contraceptive prescribed for any given patient should be that product which contains the least amount of estrogen that is compatible with an acceptable pregnancy rate and patient acceptance.

### CONTRAINDICATIONS

1. Thrombophlebitis or thromboembolic disorders
2. A past history of deep-vein thrombophlebitis or thromboembolic disorders
3. Cerebral vascular or coronary artery disease
4. Known or suspected carcinoma of the breast
5. Known or suspected estrogen-dependent neoplasia
6. Undiagnosed abnormal genital bleeding
7. Known or suspected pregnancy (See No. 5 under WARNINGS)
8. Benign or malignant liver tumor which developed during the use of oral contraceptives or other estrogen-containing products

### WARNINGS

**Cigarette smoking increases the risk of serious cardiovascular side effects from oral contraceptive use. This risk increases with age and with heavy smoking (15 or more cigarettes per day) and is quite marked in women over 35 years of age. Women who use oral contraceptives should be strongly advised not to smoke.**

The use of oral contraceptives is associated with increased risk of several serious conditions including thromboembolism, stroke, myocardial infarction, hepatic adenoma, gallbladder disease, and hypertension. Practitioners prescribing oral contraceptives should be familiar with the following information relating to these risks.

**1. Thromboembolic Disorders and Other Vascular Problems:** An increased risk of thromboembolic and thrombotic disease associated with the use of oral contraceptives is well-established. Studies have demonstrated an increased risk of fatal and nonfatal venous thromboembolism and stroke, both hemorrhagic and thrombotic.

**Cerebrovascular disorders:** In a collaborative study in women with and without predisposing causes, it was estimated that the risk of hemorrhagic stroke was 2.0 times greater in users than nonusers, and the risk of thrombotic stroke was 4.0 to 9.5 times greater.

**Myocardial infarction:** An increased risk of myocardial infarction associated with oral contraceptives has been reported confirming a previously suspected association. These studies found that the greater the number of underlying risk factors (cigarette smoking, hypertension, hypercholesterolemia, obesity, diabetes, history of preclampsia toxemia) for coronary artery disease, the higher the risk of developing myocardial infarction, regardless of whether the patient was an oral contraceptive user or not. Oral contraceptives, however, were found to be a clear additional risk factor.

It has been estimated that users who do not smoke (smoking is considered a major predisposing condition to myocardial infarction) are about twice as likely to have a fatal myocardial infarction as nonusers who do not smoke. Oral contraceptive users who are smokers have about a fivefold increased risk of fatal infarction compared to users who do not smoke, but about a tenfold to twelvefold increased risk compared to nonusers who do not smoke. The amount of smoking is also an important factor.

**Risk of dose:** In an analysis of data, British investigators concluded that the risk of thromboembolism including coronary thrombosis is directly related to the dose of estrogen used in oral contraceptives; however, the quantity of estrogen may not be the sole factor involved.

**Estimate of excess mortality from circulatory diseases:** The risk of diseases of the circulatory system is concentrated in older women, in those with a long duration of use, and in cigarette smokers.

A study of available data from a variety of sources concluded that the mortality associated with all methods of birth control is low and below that associated with childbirth, with the exception of oral contraceptives in women over 40 who smoke.

The risk of thromboembolic and thrombotic diseases associated with oral contraceptives increases with age after approximately age 30 and, for myocardial infarction, is further increased by hypertension, hypercholesterolemia, obesity, diabetes, or history of preclampsia toxemia, and especially by cigarette smoking.

The physician and the patient should be alert to the earliest manifestations of thromboembolic and thrombotic disorders. Should any occur or be suspected, the drug should be discontinued immediately.

A fourfold to sixfold increased risk of postsurgery thromboembolic complications has been reported in users. If feasible, oral contraceptives should be discontinued at least four weeks before surgery of a type associated with an increased risk of thromboembolism or prolonged immobilization.

**2. Ocular lesions:** Neuro-ocular lesions, such as optic neuritis or retinal thrombosis, have been associated with the use of oral contraceptives. Discontinue the oral contraceptive if there is unexplained sudden or gradual, partial, or complete loss of vision, onset of proptosis or diplopia, papilledema, or retinal vascular lesions.

**3. Carcinoma:** Long-term continuous administration of estrogen in certain animal species increases the frequency of carcinoma of the breast, cervix, vagina, and liver.

In humans, an increased risk of endometrial carcinoma associated with the prolonged use of exogenous estrogen in postmenopausal women has been reported. However, there is no evidence suggesting increased risk of endometrial cancer in users of conventional combination or progestogen-only oral contraceptives.

Studies found no evidence of increase in breast cancer in women taking oral contraceptives; however, an excess risk in users with documented benign breast disease was reported.

There is no confirmed evidence of users is, nevertheless, essential. In cases of undiagnosed persistent or recurrent abnormal vaginal bleeding, appropriate diagnostic measures should be taken to rule out malignancy. Women with a strong family history of breast cancer, or who have breast nodules, fibrocystic disease, or abnormal mammograms, should be monitored with particular care.

**4. Hepatic Tumors:** Benign hepatic adenomas have been found to be associated with oral contraceptives. Because hepatic adenomas may rupture and may cause death through intra-abdominal hemorrhage, they should be considered in women presenting abdominal pain and tenderness, abdominal mass, or shock.

A few cases of hepatocellular carcinoma have been reported in women taking oral contraceptives. The relationship of these drugs to this type of malignancy is not known at this time.

**5. Usage in or Immediately Preceding Pregnancy, Birth Defects in Offspring, and Malignancy in Female Offspring:** During early pregnancy, female sex hormones may seriously damage the offspring.

An increased risk of congenital anomalies, including heart defects and limb defects, has been reported with the use of oral contraceptives in pregnancy.

There is some evidence that triploidy and possibly other types of polyploidy are increased among abortuses from women who become pregnant soon after ceasing oral contraceptives.

Pregnancy should be ruled out before continuing an oral contraceptive in any patient who has missed two consecutive menstrual periods. If the patient has not missed a period, and oral contraceptive use should be considered at the time of the first missed period, and oral contraceptives should be withheld until pregnancy has been ruled out. If pregnancy is confirmed, the patient should be apprised of the potential risks to the fetus and the advisability of continuation of the pregnancy should be discussed.

Women who discontinue oral contraceptives with the intent of becoming pregnant should use an alternate form of contraception for a period of time before attempting to conceive.

Administration of progestogen-only or progestogen-estrogen combinations to induce withdrawal bleeding should not be used as a test of pregnancy.

**6. Gallbladder Disease:** Studies report an increased risk of surgically confirmed gallbladder disease in users of oral contraceptives.

**7. Carbohydrate and Lipid Metabolic Effects:** Because decreased glucose tolerance has been observed in a significant percentage of patients, prediabetic and diabetic patients should be carefully observed while receiving oral contraceptives.

An increase in triglycerides and total phospholipids has been observed.

**8. Elevated Blood Pressure:** An increase in blood pressure has been reported in patients receiving oral contraceptives. The prevalence in users increases with longer exposure. Age is also strongly correlated with development of hypertension. Women who previously have had hypertension during pregnancy may be more likely to develop elevation of blood pressure.

**9. Headache:** Onset or exacerbation of migraine or development of headache of a new pattern which is recurrent, persistent, or severe, requires discontinuation of oral contraceptives.

**10. Bleeding Irregularities:** Breakthrough bleeding, spotting, and amenorrhea are frequent reasons for patients discontinuing oral contraceptives. In breakthrough bleeding, nonfunctional causes should be borne in mind. In undiagnosed abnormal bleeding from the vagina, adequate diagnostic measures are indicated to rule out pregnancy or malignancy.

Women with a past history of oligomenorrhea or secondary amenorrhea, or young women without regular cycles should be advised that they may have a tendency to remain anovulatory or to become amenorrheic after discontinuation of oral contraceptives.

**11. Ectopic Pregnancy:** Ectopic as well as intrauterine pregnancy may occur in contraceptive failures.

**12. Breast Feeding:** Oral contraceptives may interfere with lactation. Furthermore, a small fraction of the hormonal agents in oral contraceptives has been identified in the milk of mothers receiving these drugs.

### PRECAUTIONS

1. A complete medical and family history should be taken prior to the initiation of oral contraceptives. The pretreatment and periodic physical examinations should include special reference to blood pressure, breasts, abdomen, and pelvic organs, including Papanicolaou smear and relevant laboratory tests. As a general rule, oral contraceptives should not be prescribed for longer than one year without another examination.

2. Preexisting uterine leiomyomata may increase in size.

3. Patients with a history of psychic depression should be carefully observed and the drug discontinued if depression recurs to a serious degree.

4. Oral contraceptives may cause fluid retention and should be prescribed with caution, and only with careful monitoring, in patients with conditions which might be aggravated.

5. Patients with a past history of jaundice during pregnancy have an increased risk of recurrence of jaundice. If jaundice develops, the medication should be discontinued.

6. Steroid hormones may be poorly metabolized and should be administered with caution in patients with impaired liver function.

7. Users may have disturbances in normal tryptophan metabolism, which may result in a relative pyridoxine deficiency.

8. Serum folate levels may be depressed.

9. The pathologist should be advised of oral contraceptive therapy when relevant specimens are submitted.

10. Certain endocrine and liver function tests and blood components may be affected.

(a) Increased sulfobromophthalein retention. (b) Increased prothrombin and factors VII, VIII, IX, and X, decreased antithrombin 3, increased norepinephrine-induced platelet aggregability. (c) Increased thyroid-binding globulin (TBG) leading to increased circulating total thyroid hormone. (d) Decreased pregnandiol excretion. (e) Reduced response to metyrapone test.

**Drug Interactions:** Reduced efficacy and increased incidence of breakthrough bleeding have been associated with concomitant use of rifampin. A similar association has been suggested with barbiturates, phenylbutazone, phenytoin sodium, tetracycline, and ampicillin.

### ADVERSE REACTIONS

An increased risk of the following serious adverse reactions has been associated with oral contraceptives:

Thrombophlebitis, Pulmonary embolism, Coronary thrombosis, Cerebral thrombosis, Cerebral hemorrhage, Hypertension, Gallbladder disease, Benign hepatomas, Congenital anomalies.

There is evidence of an association between the following conditions and the use of oral contraceptives, although additional confirmatory studies are needed: Mesenteric thrombosis, neuro-ocular lesions, eg. retinal thrombosis and optic neuritis.

The following adverse reactions have been reported in patients receiving oral contraceptives and are believed to be drug-related: Nausea and/or vomiting, usually the most common adverse reactions, occur in approximately 10 percent or less of patients during the first cycle. Other reactions, as a general rule, are seen much less frequently or only occasionally: gastrointestinal symptoms; breakthrough bleeding; spotting; change in menstrual flow; dysmenorrhea; amenorrhea during and after treatment; temporary infertility after discontinuance of treatment; edema; chloasma or melasma; breast changes; change in weight; change in cervical erosion and cervical secretion; possible diminution in lactation when given immediately postpartum; cholestatic jaundice; migraine; increase in size of uterine leiomyomata; rash (allergic); mental depression; reduced tolerance to carbohydrates; vaginal candidiasis; change in corneal curvature; intolerance to contact lenses.

The following adverse reactions have been reported and the association has been neither confirmed nor refuted.

Premenstrual-like syndrome; cataracts; changes in libido; chorea; changes in appetite; cystitis-like syndrome; headache; nervousness; dizziness; hirsutism; loss of scalp hair; erythema multiforme; erythema nodosum; hemorrhagic eruption; vaginitis; porphyria.

### Special Notes on Administration

Loestrin **[21]** 1.5/30 (1.5 mg norethindrone acetate and 30 mcg ethinyl estradiol tablets, USP)—Menstruation usually begins two or three days, but may begin as late as the fourth or fifth day, after discontinuing medication.

Loestrin **[28]** 1.5/30—Menstruation usually begins two or three days, but may begin as late as the fourth or fifth day, after the brown ferrous fumarate tablets have been started.

Because of the relatively low estrogenic content, Loestrin 1.5/30 is not a good cyclic regulator. There are patients whose inherent hormone balance will require larger amounts of estrogen than that contained in Loestrin 1.5/30 to achieve cyclic regularity. These patients experience altered bleeding patterns, which do not conform to treatment schedules.

The physician should be alert to the fact that the irregular bleeding patterns could mask bleeding from organic cause, and appropriate diagnostic measures should be taken if the bleeding persists or continues after changing to a higher estrogen-content product.

After several months, bleeding may be reduced to a virtual absence; reduced flow may be a result of medication and not indicative of pregnancy.

### HOW SUPPLIED

Loestrin **[21]** 1.5/30 is available in compacts each containing 21 green tablets and 7 brown tablets. Each green tablet contains 1.5 mg of norethindrone acetate and 30 mcg of ethinyl estradiol. Each brown tablet contains 75 mg of ferrous fumarate. Available in packages of five compacts and packages of five refills.

Loestrin **[28]** 1.5/30 is available in compacts each containing 21 tablets. Each green tablet contains 1.5 mg of norethindrone acetate and 30 mcg of ethinyl estradiol. Available in packages of five compacts and packages of five refills.

September 1980

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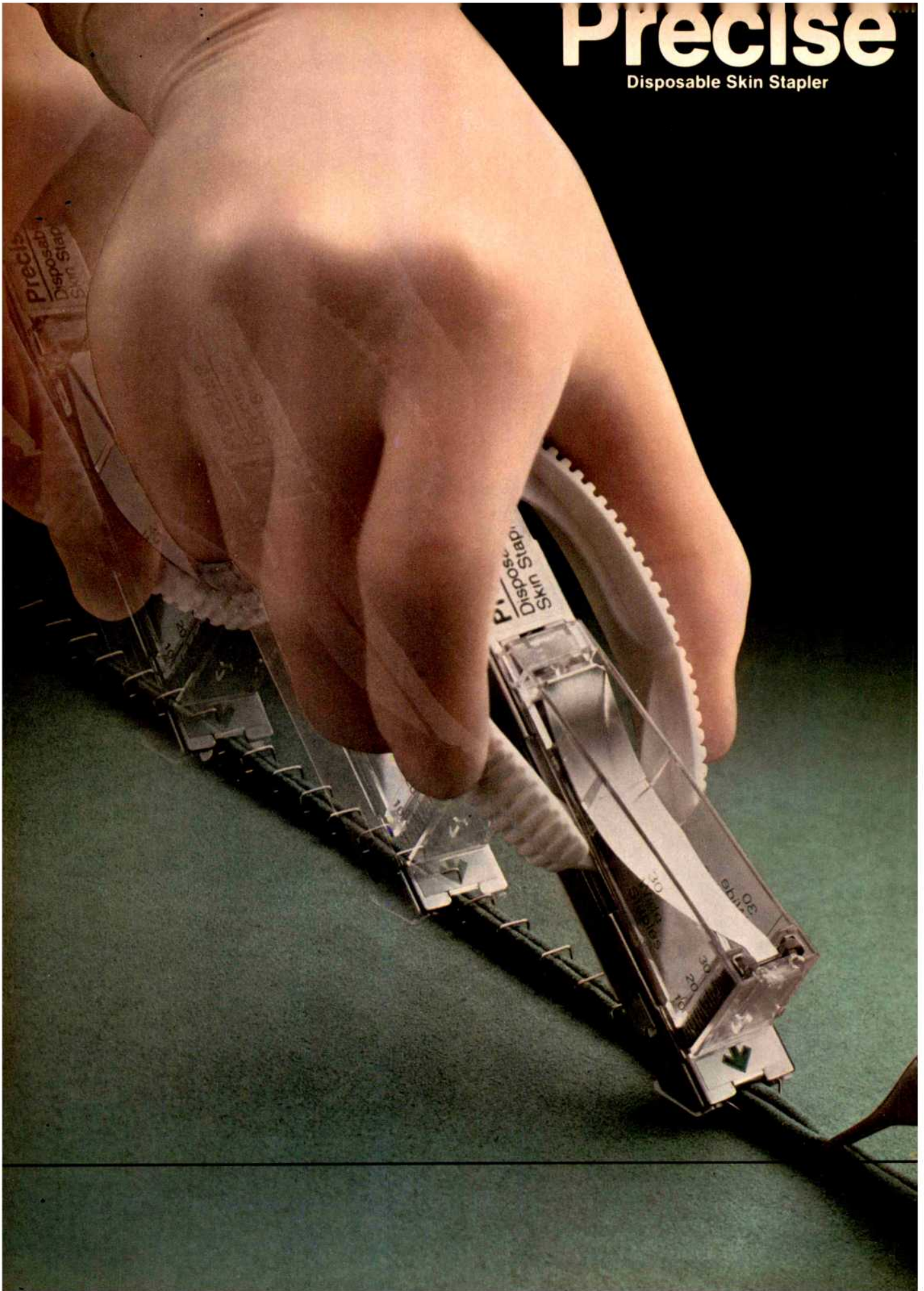
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|                                                                                            |                           | <i>Oval</i> -----                                                          | 22, 23, 24            |

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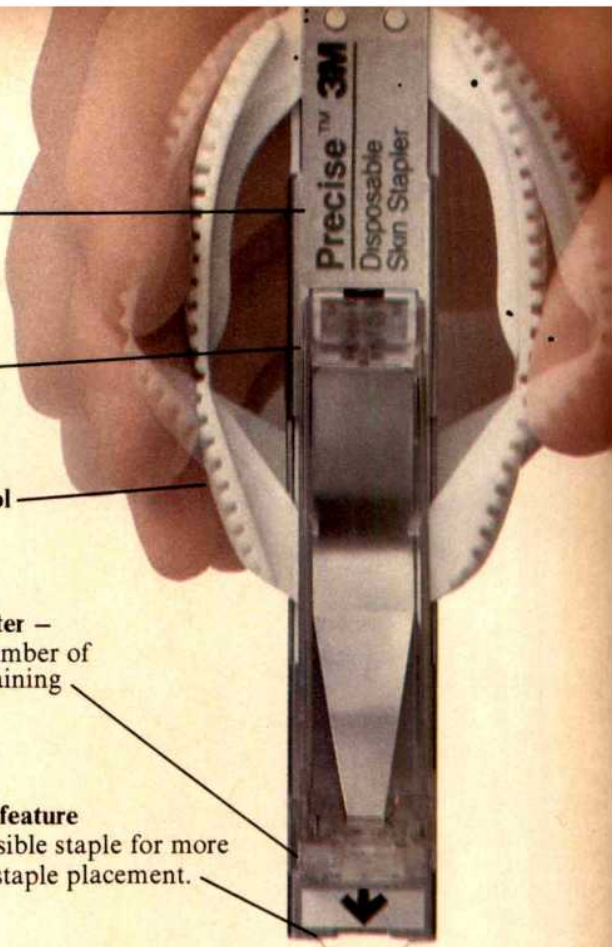
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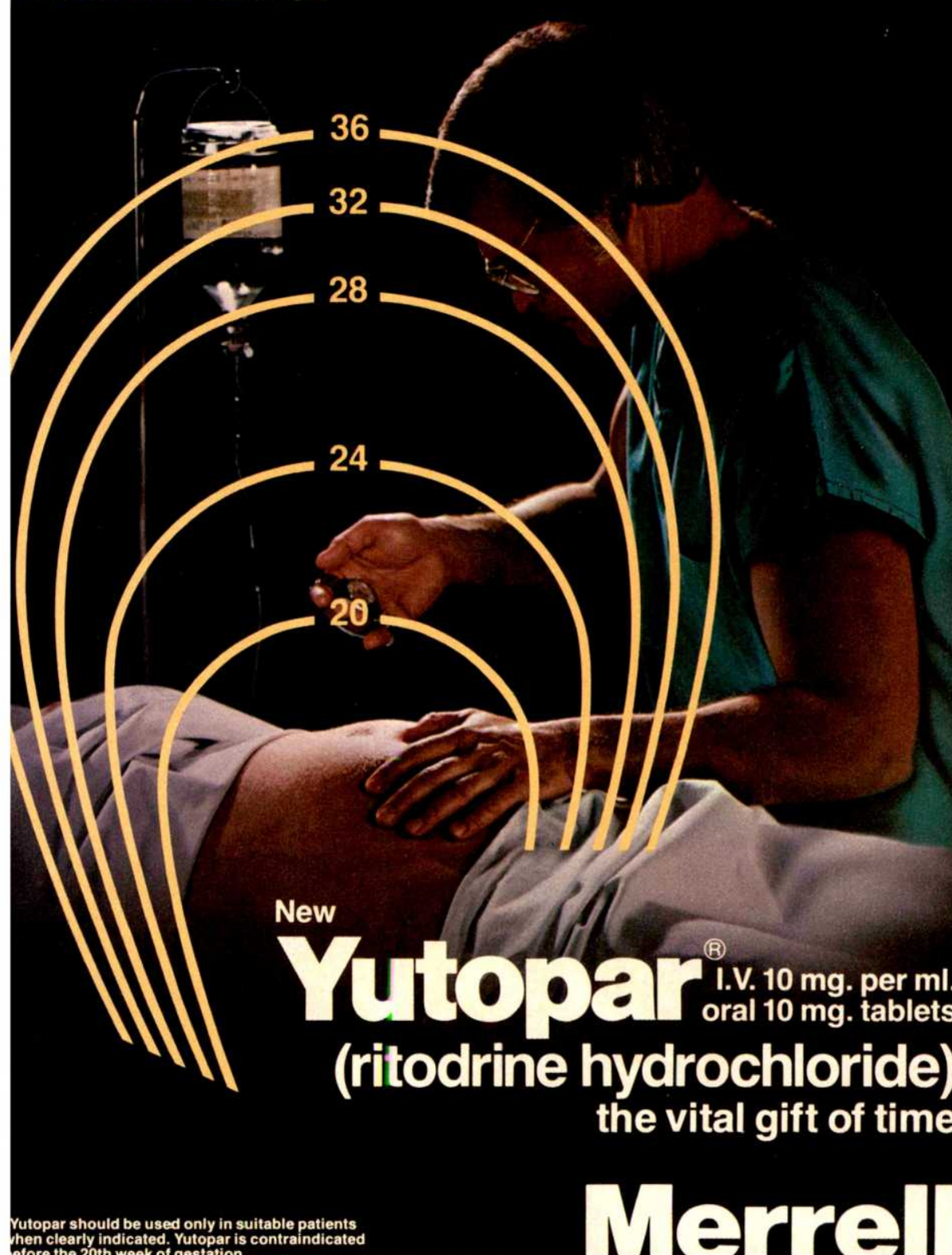
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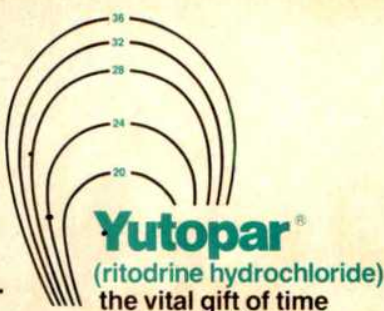


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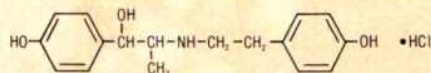


CAUTION: Federal law prohibits dispensing without prescription.

#### DESCRIPTION

Yutopar, which contains the betamimetic (beta sympathomimetic amine) ritodrine hydrochloride, is available in two dosage forms. Yutopar for parenteral (intravenous) use is a clear, colorless, sterile, aqueous solution; each milliliter contains 10 mg. of ritodrine hydrochloride, 0.44% (w/v) of acetic acid, 0.24% (w/v) of sodium hydroxide, 0.1% (w/v) of sodium metabisulfite, and 0.29% (w/v) of sodium chloride in water for injection USP. Hydrochloric acid or additional sodium hydroxide is used to adjust pH. Each Yutopar tablet contains 10 mg. of ritodrine hydrochloride.

Ritodrine hydrochloride is a white, odorless crystalline powder, freely soluble in water, with a melting point between 196° and 205° C. The chemical name of the ritodrine hydrochloride is erythro- $\beta$ -hydroxy- $\alpha$ -[1-[(p-hydroxyphenyl)ethyl]amino]benzyl alcohol hydrochloride and has the chemical structure:



#### CLINICAL PHARMACOLOGY

Yutopar (ritodrine hydrochloride) is a beta-receptor agonist, which has been shown by *in vitro* and *in vivo* pharmacologic studies in animals to exert a preferential effect on the  $\beta_2$  adrenergic receptors such as those in the uterine smooth muscle. Stimulation of the  $\beta_2$  receptors inhibits contractility of the uterine smooth muscle.

In humans, intravenous infusions of 0.05 to 0.30 mg./min. or single oral doses of 10 to 20 mg. decreased the intensity and frequency of uterine contractions. These effects were antagonized by beta-blocking compounds. Intravenous administration induced an immediate dose-related elevation of heart rate with maximum mean increases between 19 and 40 beats per minute. Widening of the pulse pressure was also observed; the average increase in systolic blood pressure was 4.0 mm. Hg, and the average decrease in diastolic pressure was 3.2 mm. Hg. With oral intake, the increase in heart rate was mild and delayed.

During intravenous infusion in humans, transient elevations of blood glucose, insulin, and free fatty acids have been observed. Decreased serum potassium has also been found, but effects on other electrolytes have not been reported.

Serum kinetics in humans (non-pregnant females) of an intravenous infusion of 60 minutes duration were determined by measuring serum ritodrine levels by a radioimmunoassay technique. Three half-lives were calculated: the first of 6 to 9 minutes dominated the ascending phase of the serum drug-level curve, the second phase of 1.7 to 2.6 hours dominated the descending curve, and finally, a third phase, with a half-life of more than 10 hours, was discernible. In a study of serum kinetics after oral ingestion (male subjects), the decline of serum drug levels could be described in terms of a two-phase decay with an initial half-life of 1.3 hours and a final half-life of 12 hours. With either route of administration, 90% of the excretion was completed within 24 hours after the dose.

Comparison of ritodrine serum levels after intravenous administration with those after oral dosage indicates the oral bioavailability is about 30%. Intravenous infusion at a rate of 0.15 mg./min. for 1 hour yielded maximum serum levels ranging between 32 and 52 ng./ml. in a group of 6 non-pregnant female volunteers; maximum serum levels following single and repeated (4 x 10 mg./24 hr.) 10 mg. oral doses ranged between 5 and 15 ng./ml. and were obtained within 30 to 60 minutes after ingestion.

Placental transfer was confirmed by measurement of drug concentrations in cord blood showing that ritodrine and its conjugates reach the fetal circulation.

#### INDICATIONS AND USAGE

Yutopar is indicated for the management of preterm labor in suitable patients.

Administered intravenously, the drug will decrease uterine activity and thus prolong gestation in the majority of such patients. After intravenous Yutopar has arrested the acute episode, oral administration may help to avert relapse. Additional acute episodes may be treated by repeating the intravenous infusion. The incidence of neonatal mortality and respiratory distress syndrome increases when the normal gestation period is shortened.

Since successful inhibition of labor is more likely with early treatment, therapy with Yutopar should be instituted as soon as the diagnosis of preterm labor is established and contraindications ruled out in pregnancies of 20 or more weeks' gestation. The efficacy and safety of Yutopar in advanced labor, that is, when cervical dilatation is more than 4 cm. or effacement is more than 80%, have not been established.

#### CONTRAINDICATIONS

Yutopar is contraindicated before the 20th week of pregnancy.

Yutopar is also contraindicated in those conditions of the mother or fetus in which continuation of pregnancy is hazardous; specific contraindications include:

1. Antepartum hemorrhage which demands immediate delivery
2. Eclampsia and severe preeclampsia
3. Intrauterine fetal death
4. Chorioamnionitis
5. Maternal cardiac disease
6. Pulmonary hypertension
7. Maternal hyperthyroidism
8. Uncontrolled maternal diabetes mellitus (See Precautions.)
9. Pre-existing maternal medical conditions that would be seriously affected by the known pharmacologic properties of a betamimetic drug, such as: hypovolemia, cardiac arrhythmias associated with tachycardia or digitalis intoxication, uncontrolled hypertension, pheochromocytoma, bronchial asthma already treated by betamimetics and/or steroids
10. Known hypersensitivity to any component of the product

#### WARNINGS

Maternal pulmonary edema has been reported rarely in patients treated concomitantly with Yutopar and corticosteroids and one maternal death has been reported in such a patient. Patients so treated must be closely monitored in hospital. If pulmonary edema develops, the drugs should be discontinued and the edema managed by conventional means.

Intravenous administration of Yutopar should be supervised by persons having knowledge of the pharmacology of the drug and who are qualified to identify and manage complications of drug administration and pregnancy. Because cardiovascular responses are common and more pronounced during intravenous administration of Yutopar, cardiovascular effects, including maternal pulse rate and blood pressure and fetal heart rate, should be closely monitored. Care should be exercised for maternal signs and symptoms of pulmonary edema. Occult cardiac disease may be unmasked with the use of Yutopar.

The drug should not be administered to patients with mild to moderate preeclampsia, hypertension, or diabetes unless the attending physician considers that the benefits clearly outweigh the risks.

#### PRECAUTIONS

When Yutopar is used for the management of preterm labor in a patient with premature rupture of the membranes, the benefits of delaying delivery should be balanced against the potential risks of development of chorioamnionitis.

Among low birth weight infants, approximately 9% may be growth retarded for gestational age. Therefore, Intra-Uterine Growth Retardation (IUGR) should be considered in the differential diagnosis of preterm labor; this is especially important when the gestational age is in doubt. The decision to continue or reinstitute the administration of Yutopar will depend on an assessment of fetal maturity. In addition to clinical parameters, other studies, such as sonography or amniocentesis, may be helpful in establishing the state of fetal maturity if it is in doubt.

#### Laboratory Tests

Because intravenous administration of Yutopar has been shown to elevate plasma insulin and glucose and to decrease plasma potassium concentrations, monitoring of glucose and electrolyte levels is recommended during protracted infusions. Special attention should be paid to biochemical variables when treating diabetic patients or those receiving potassium-depleting diuretics.

#### Drug Interactions

Corticosteroids used concomitantly may lead to pulmonary edema. (See Warnings.)

The effects of other sympathomimetic amines may be potentiated when concurrently administered and these effects may be additive. A sufficient time interval should elapse prior to administration of another sympathomimetic drug.

Beta-adrenergic blocking drugs inhibit the action of Yutopar; coadministration of these drugs should, therefore, be avoided.

With anesthetics used in surgery, the possibility that hypotensive effects may be potentiated should be considered.

#### Carcinogenesis

In rats given oral doses of 1, 10, and 150 mg./kg./day of ritodrine hydrochloride for 82 weeks, no evidence of tumorigenicity was noted.

#### Pregnancy

##### Teratogenic Effects

(Pregnancy Category B)

Reproduction studies were performed in rats and rabbits. The doses employed intravenously were 1/9 (1 mg./kg.), 1/3 (3 mg./kg.), and 1 (9 mg./kg.) times the maximum human daily intravenous dose (but given to the animals as a bolus rather than by infusion). The oral doses, 10 and 100 mg./kg. represented 5 and 50 times the maximum human daily oral maintenance dose. The results of these studies have revealed no evidence of impaired fertility or harm to the fetus due to ritodrine hydrochloride.

No adverse fetal effects were encountered when single intravenous doses of 1, 3, and 9 mg./kg./day or oral doses of 10 and 100 mg./kg./day were given to rats and rabbits on Days 6 through 15 and 6 through 18 of gestation, respectively. Intravenous doses of 1 and 8 mg./kg./day or oral doses of 10 and 100 mg./kg./day administered to the mother from Day 15 of pregnancy to Day 21 postpartum did not affect perinatal or postnatal development in rats. A slight increase in fetal weight in the rat was observed. Oral administration to both sexes did not impair fertility or reproductive performance. Lethal doses to pregnant rats did not cause immediate fetal demise. There are no adequate and well-controlled studies of Yutopar effects in pregnant women before 20 weeks' gestation; therefore, this drug should not be used before the 20th week of pregnancy. Studies of Yutopar administered to pregnant women from the 20th week of gestation have not shown increased risk of fetal abnormalities. Follow-up of selected variables in a small number of children for up to 2 years has not revealed harmful effects on growth, developmental or functional maturation. Nonetheless, although clinical studies did not demonstrate a risk of permanent adverse fetal effects from Yutopar, the possibility cannot be excluded; therefore, Yutopar should be used only when clearly indicated.

Some studies indicate that infants born before 36 weeks' gestation make up less than 10% of all births but account for as many as 75% of perinatal deaths and one-half of all neurologically handicapped infants. There are data available indicating that infants born at any time prior to full term may manifest a higher incidence of neurologic or other handicaps than occurs in the total population of infants born at or after full term. In delaying or preventing preterm labor, the use of Yutopar should result in an overall increase in neonatal survival. Handicapped infants who might not have otherwise survived may survive.

#### ADVERSE REACTIONS

The unwanted effects of Yutopar are related to its betamimetic activity and usually are controlled by suitable dosage adjustment.

##### Effects Associated with Intravenous Administration

(Usual effects (80-100% of patients))

Intravenous infusion of Yutopar leads almost invariably to dose-related alterations in maternal and fetal heart rates and in maternal blood pressure. During clinical studies in which the maximum infusion rate was limited to 0.35 mg./min. (one patient received 0.40 mg./min.), the maximum maternal and fetal heart rates averaged, respectively, 130 (range 60 to 180) and 164 (range 130 to 200) beats per minute. The maximum maternal systolic blood pressures averaged 128 mm. Hg (range 96 to 162 mm. Hg), an average increase of 12 mm. Hg from pretreatment levels. The minimum maternal diastolic blood pressures averaged 48 mm. Hg (range 0 to 76 mm. Hg), an average decrease of 23 mm. Hg from pretreatment levels. While the more severe effects were usually managed effectively by dosage adjustments, in less than 1% of patients, persistent maternal tachycardia or decreased diastolic blood pressure required withdrawal of the drug.

Yutopar infusion is associated with transient elevation of blood glucose and insulin, which decreases toward normal values after 48 to 72 hours despite continued infusion. Elevation of free fatty acids and cAMP has been reported. Reduction of potassium levels should be expected; other biochemical effects have not been reported.

##### Frequent effects (10-50% of patients)

Intravenous Yutopar, in about one-third of the patients, was associated with palpitation, tremor, nausea, vomiting, headache, or erythema was observed in 10 to 15% of patients.

##### Occasional effects (5-10% of patients)

Nervousness, jitteriness, restlessness, emotional upset, or anxiety was reported in 5 to 6% of patients and malaise in similar numbers.

##### Inrequent effects (1-3% of patients)

Cardiac symptoms including chest pain or tightness (rarely associated with abnormalities of ECG) and arrhythmia were reported in 1 to 2% of patients.

Other infrequently reported maternal effects included: anaphylactic shock, rash, heart murmur, epigastric distress, ileus, bloating, constipation, diarrhea, dyspnea, hyperventilation, hemolytic icterus, glycosuria, lactic acidosis, sweating, chills, drowsiness, and weakness.

#### Neonatal Effects

Infrequently reported neonatal symptoms include hypoglycemia and ileus. In addition, hypocalcemia and hypotension have been reported in neonates whose mothers were treated with other betamimetic agents.

#### Effects Associated with Oral Administration

##### Frequent effects (<50% of patients)

Oral ritodrine in clinical studies was often associated with small increases in maternal heart rate, but little or no effect upon either maternal systolic or diastolic blood pressure or upon fetal heart rate was found.

Oral ritodrine in 10 to 15% of patients was associated with palpitation or tremor. Nausea and jitteriness were less frequent (5 to 8%), while rash was observed in some patients (3 to 4%), and arrhythmia was infrequent (about 1%).

#### OVERDOSAGE

The symptoms of overdosage are those of excessive beta-adrenergic stimulation including exaggeration of the known pharmacologic effects, the most prominent being tachycardia (maternal and fetal), palpitation, cardiac arrhythmia, hypotension, dyspnea, nervousness, tremor, nausea, and vomiting. If an excess of ritodrine tablets is ingested, gastric lavage or induction of emesis should be carried out followed by administration of activated charcoal. When symptoms of overdose occur as a result of intravenous administration, ritodrine should be discontinued, an appropriate beta-blocking agent may be used as an antidote. Ritodrine hydrochloride is dialyzable.

Acute intravenous toxicity was studied in rats and rabbits and acute oral toxicity in mice, rats, guinea pigs, and dogs. The LD50 values in the most sensitive of the species used were 64 mg./kg. intravenously in the nonpregnant rabbit and 540 mg./kg. orally in the nonpregnant mouse. The intravenous LD50 value in the pregnant rat was 85 mg./kg. The amount of drug required to produce symptoms of overdose in humans is individually variable. No reports of human mortality due to overdose have been received.

#### DOSEAGE AND ADMINISTRATION

In the management of preterm labor, the initial intravenous treatment should usually be followed by oral administration. The optimum dose of Yutopar is determined by a clinical balance of uterine response and unwanted effects.

##### Intravenous Therapy

Do not use intravenous Yutopar if the solution is discolored or contains any precipitate or particulate matter.

Yutopar solution should be used promptly after preparation, but in no case after 48 hours of preparation.

**Method of Administration:** To minimize the risks of hypotension, the patient should be maintained in the left lateral position during infusion and careful attention given to her hydration.

For appropriate control and dose titration, a controlled infusion device is recommended to adjust the rate of flow in drops/minute. An i.v. microdrip chamber (60 drops/ml.) can provide a convenient range of infusion rates within the recommended dose range for Yutopar.

**Recommended Dilution:** 150 mg. ritodrine hydrochloride (3 ampuls) in 500 ml. fluid yielding a final concentration of 0.3 mg./ml. Ritodrine for intravenous infusion should be diluted with one of the following:

- 0.9% w/v sodium chloride solution
- 5% w/v dextrose solution
- 10% w/v dextran 40 in 0.9% w/v sodium chloride solution
- 10% w/v invert sugar solution
- Compound sodium chloride solution (Ringer's solution)
- Hartmann's solution

Intravenous therapy should be started as soon as possible after diagnosis. The usual initial dose is 0.1 mg./minute (0.33 ml./min., 20 drops/min. using a microdrip chamber at the recommended dilution), to be gradually increased according to the results by 0.05 mg./minute (0.17 ml./min., 10 drops/min. using a microdrip chamber at the recommended dilution) every 10 minutes until the desired result is attained. The effective dosage usually lies between 0.15 and 0.35 mg./minute (0.50 to 1.17 ml./min., 30-70 drops/min. using a microdrip chamber at the recommended dilution). The infusion should generally be continued for at least 12 hours after uterine contractions cease. With the recommended dilution, the maximum volume of fluid that might be administered after 12 hours at the highest dose (0.35 mg./min.) will be approximately 840 ml. The amount of i.v. fluids administered and the rate of administration should be monitored to avoid circulatory fluid overload. Frequent monitoring of maternal uterine contractions, heart rate, and blood pressure, and of fetal heart rate is required, with dosage individually titrated according to response.

##### Oral Maintenance

One tablet (10 mg.) may be given approximately 30 minutes before the termination of intravenous therapy. The usual dosage schedule for the first 24 hours of oral maintenance is 1 tablet (10 mg.) every two hours. Thereafter the usual dose is 1 or 2 tablets (10 to 20 mg.) every four to six hours, the dose depending on uterine activity and unwanted effects. The total daily dose of oral ritodrine should not exceed 120 mg. The treatment may be continued as long as the physician considers it desirable to prolong pregnancy.

Recurrence of unwanted preterm labor may be treated with repeated infusion of Yutopar.

#### HOW SUPPLIED

NDC 0068-0599-53: 5 ml. ampuls in boxes of 12. Each ampul contains 50 mg. (10 mg./ml.) of ritodrine hydrochloride.

NDC 0068-0595-60: 10 mg. tablets in bottles of 60. Each round, yellow tablet contains 10 mg. ritodrine hydrochloride and is inscribed YUTOPAR on one side and scored on the other.

Product Information as of October, 1980

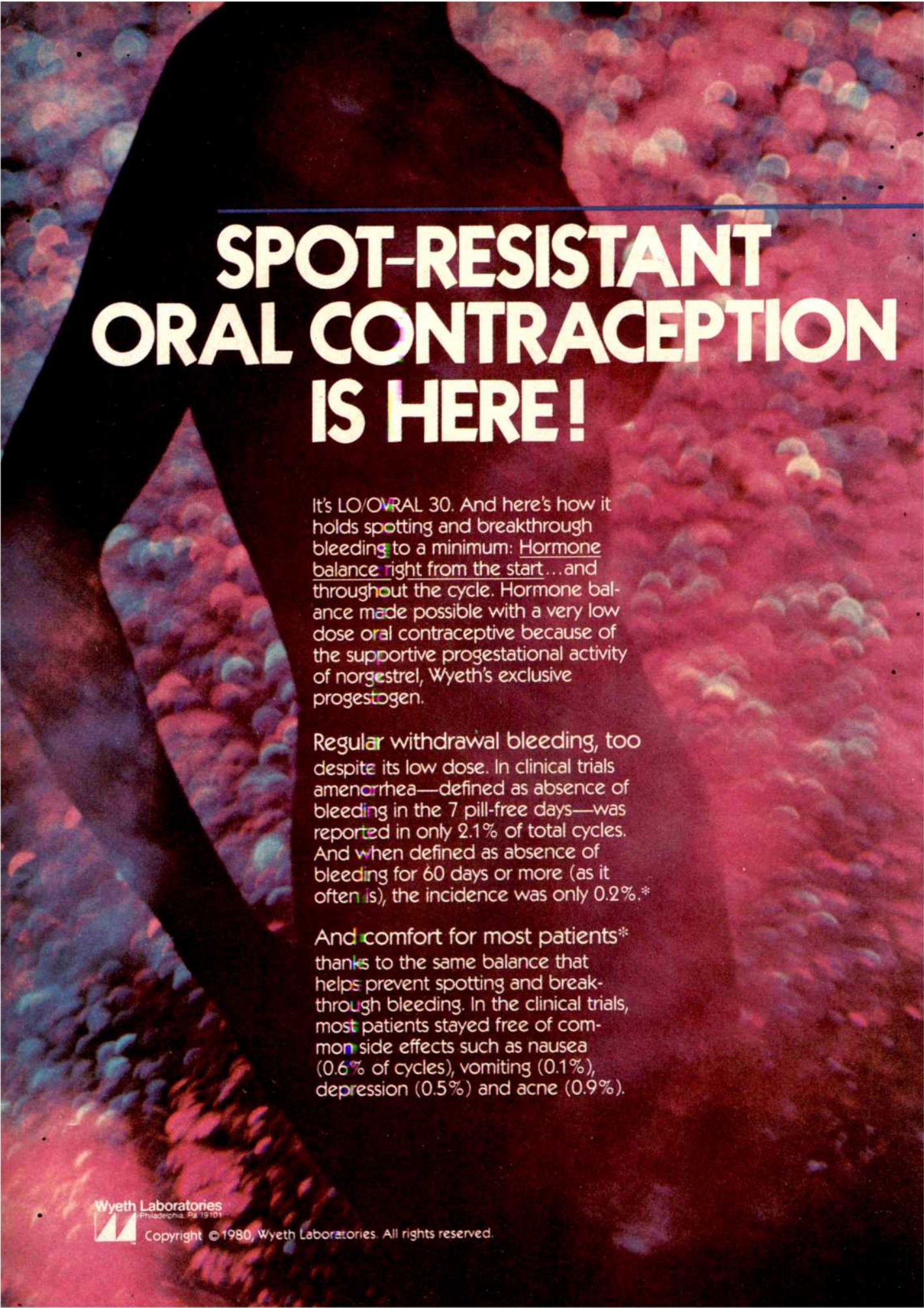
Both the tablet and intravenous dosage forms should be stored at room temperature, preferably below 86°F (30°C.). Protect from excessive heat.

Parenteral (intravenous) dosage form manufactured by TAYLOR PHARMACEUTICAL COMPANY, Decatur, Illinois 62525. Distributed by MERRELL-NATIONAL LABORATORIES, Division of Richardson-Merrell Inc., Cincinnati, Ohio 45215, U.S.A. Yutopar® licensed by Duphar B.V., Amsterdam, Holland

**Merrell**

O-6234 (Y068B)





# SPOT-RESISTANT ORAL CONTRACEPTION IS HERE!

It's LO/OVRAL 30. And here's how it holds spotting and breakthrough bleeding to a minimum: Hormone balance right from the start...and throughout the cycle. Hormone balance made possible with a very low dose oral contraceptive because of the supportive progestational activity of norgestrel, Wyeth's exclusive progestogen.

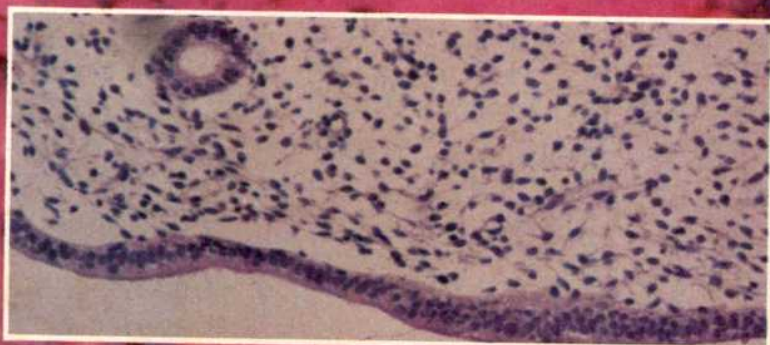
Regular withdrawal bleeding, too despite its low dose. In clinical trials amenorrhea—defined as absence of bleeding in the 7 pill-free days—was reported in only 2.1% of total cycles. And when defined as absence of bleeding for 60 days or more (as it often is), the incidence was only 0.2%.\*

And comfort for most patients\* thanks to the same balance that helps prevent spotting and breakthrough bleeding. In the clinical trials, most patients stayed free of common side effects such as nausea (0.6% of cycles), vomiting (0.1%), depression (0.5%) and acne (0.9%).



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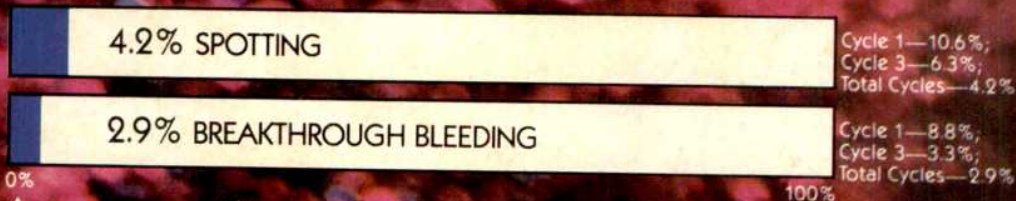


#### LO/OVRAL—1st cycle

Biopsy of intact endometrium in stable, progestogen suppressed state. Small, tubular gland in loose stroma without hemorrhage, fragmentation or debris characteristic of desquamating endometrium.

Even in the first cycle, the effective action of LO/OVRAL on the endometrium, such as you see here, provides the resistance against spotting and breakthrough bleeding you hope to achieve.

## HISTOLOGICALLY... AND CLINICALLY.



A near-spotless record—  
in clinical trials involving 22,489 cycles\*

\*Serious as well as minor adverse reactions have been reported following the use of all oral contraceptives. Contraindications, Warnings, Precautions, Adverse Reactions, etc., in the accompanying In Brief should be carefully considered.

See full prescribing information.

# LO/OVRAL<sup>®</sup> 30 mcg

each tablet contains 0.3 mg norgestrel with 0.03 mg ethinyl estradiol, Wyeth

...with a near-spotless record!

See important information on following page.



**Contraindications**—OC's should not be used in women with any of the following conditions: 1. Thrombophlebitis or thromboembolic disorders. 2. A past history of deep-vein thrombophlebitis or thromboembolic disorders. 3. Cerebral-vascular or coronary-artery disease. 4. Known or suspected carcinoma of the breast. 5. Known or suspected estrogen-dependent neoplasia. 6. Undiagnosed abnormal genital bleeding. 7. Known or suspected pregnancy (see Warning No. 5). 8. Benign or malignant liver tumor which developed during use of OC's or other estrogen-containing products.

#### Warnings

**Cigarette smoking increases the risk of serious cardiovascular side effects from oral contraceptive use. This risk increases with age and with heavy smoking (15 or more cigarettes per day) and is quite marked in women over 35 years of age. Women who use oral contraceptives should be strongly advised not to smoke.**

The use of oral contraceptives is associated with increased risk of several serious conditions, including thromboembolism, stroke, myocardial infarction, hepatic adenoma, gallbladder disease, hypertension. Practitioners prescribing oral contraceptives should be familiar with the following information relating to these risks.

**1. Thromboembolic Disorders and Other Vascular Problems**—An increased risk of thromboembolic and thrombotic disease associated with use of OC's is well established. Three principal studies in Great Britain and 3 in the U.S. have demonstrated increased risk of fatal and nonfatal venous thromboembolism and stroke, both hemorrhagic and thrombotic. These studies estimate that users of OC's are 4 to 11 times more likely than nonusers to develop these diseases without evident cause.

**CEREBROVASCULAR DISORDERS**—In a collaborative American study of cerebrovascular disorders in women with and without predisposing causes, it was estimated that the risk of hemorrhagic stroke was 2.0 times greater in users than nonusers and the risk of thrombotic stroke was 4 to 9.5 times greater in users than in nonusers.

**MYOCARDIAL INFARCTION (MI)**—An increased risk of MI associated with use of OC's has been reported, confirming a previously suspected association. These studies, conducted in the UK, found, as expected, that the greater the number of underlying risk factors for coronary artery disease (cigarette smoking, hypertension, hypercholesterolemia, obesity, diabetes, history of pre-eclampsia toxemia) the higher the risk of developing MI, regardless of whether the patient was an OC user or not. OC's, however, were found to be a clear additional risk factor. In terms of relative risk, it has been estimated that OC users who do not smoke (smoking is considered a major predisposing condition to MI) are about twice as likely to have a fatal MI as nonusers who do not smoke. OC users who are also smokers have about a 5-fold increased risk of fatal MI compared to users who do not smoke, but about a 10- to 12-fold increased risk compared to nonusers who do not smoke. Furthermore, amount of smoking is also an important factor. In determining importance of these relative risks, however, baseline rates for various age groups must be given serious consideration. Importance of other predisposing conditions mentioned above in determining relative and absolute risks has not as yet been quantified, quite likely the same synergistic action exists, but perhaps to a lesser extent.

**RISK OF DOSE**—In an analysis of data derived from several national adverse-reaction reporting systems, British investigators concluded that risk of thromboembolism, including coronary thrombosis, is directly related to dose of estrogen in OC's. Preparations containing 100 mcg or more of estrogen were associated with higher risk of thromboembolism than those containing 50-80 mcg. Their analysis did suggest, however, that quantity of estrogen may not be the sole factor involved. This finding has been confirmed in the U.S.

**ESTIMATE OF EXCESS MORTALITY FROM CIRCULATORY DISEASES**—A large prospective study carried out in the UK estimated the mortality rate per 100,000 women per year from diseases of the circulatory system for users and nonusers of OC's according to age, smoking habits, and duration of use. Overall excess death rate annually from circulatory diseases for OC users was estimated to be 20 per 100,000 (ages 15-34—5,100,000; ages 35-44—33,100,000; ages 45-49—140,100,000), risk being concentrated in older women, in those with long duration of use, and in cigarette smokers. It was not possible, however, to examine interrelationships of age, smoking, and duration of use, nor to compare effects of continuous vs. intermittent use. Although the study showed a 10-fold increase in death due to circulatory diseases in users for 5 or more years, all these deaths occurred in women 35 or older. Until larger numbers of women under 35 with continuous use for 5 or more years are available, it is not possible to assess magnitude of relative risk for this younger group. Available data from a variety of sources have been analyzed to estimate risk of death associated with various methods of contraception. Estimates of risk of death for each method include combined risk of contraceptive method (e.g., thromboembolic and thrombotic disease in the case of OC's) plus risk attributable to pregnancy or abortion in event of method failure. This latter risk varies with effectiveness of method. The study concluded that mortality associated with all methods of birth control is low and below that associated with childbirth, with the exception of OC's in women over 40 who smoke. Lowest mortality is associated with condom or diaphragm backed up by early abortion. Risk of thromboembolic and thrombotic disease associated with OC's increases with age after about 30 and, for MI, is further increased by hypertension, hypercholesterolemia, obesity, diabetes, or history of pre-eclampsia toxemia, and especially cigarette smoking. Physician and patient should be alert to earliest manifestations of thromboembolic and thrombotic disorders (e.g., thrombophlebitis, pulmonary embolism, cerebrovascular insufficiency, coronary occlusion, retinal thrombosis, and mesenteric thrombosis). Should any of these occur or be suspected, the drug should be discontinued immediately. A 4- to 6-fold increased risk of postsurgery thromboembolic complications has been reported in OC users.

1. If feasible, OC's should be discontinued at least 4 weeks before surgery of a type associated with increased risk of thromboembolism or prolonged immobilization.

2. **Ocular Lesions**—There have been reports of neuro-ocular lesions such as optic neuritis or retinal thrombosis associated with use of OC's. Discontinue OC's if there is unexplained, sudden or gradual, partial or complete loss of vision; onset of proptosis or diplopia; papilledema; or retinal-vascular lesions, and institute appropriate diagnostic and therapeutic measures.

3. **Carcinoma**—Long-term continuous administration of either natural or synthetic estrogen in certain animal species increases frequency of carcinoma of the breast, cervix, vagina, and liver. Certain synthetic progestogens, none currently contained in OC's, have been noted to increase incidence of mammary nodules, benign

physicians to a registry of cases of adenocarcinoma of the endometrium in women under 40 on OC's. Of cases found in women without predisposing risk factors (e.g., irregular bleeding at the time OC's were first given, polycystic ovaries), nearly all occurred in women who had used a sequential OC. These are no longer marketed. No evidence has been reported suggesting increased risk of endometrial cancer in users of conventional combination or progestogen-only OC's. Several studies have found no increase in breast cancer in women taking OC's or estrogens. One study, however, while also noting no overall increased risk of breast cancer in women on OC's, found an excess risk in subgroups of OC users with documented benign breast disease. Reduced occurrence of benign breast tumors in users of OC's has been well documented. In summary, there is at present no confirmed evidence from human studies of increased risk of cancer associated with OC's. Close clinical surveillance of all women on OC's is, nevertheless, essential. In all cases of undiagnosed persistent or recurrent abnormal vaginal bleeding, appropriate diagnostic measures should be taken to rule out malignancy. Women with a strong family history of breast cancer or with breast nodules, fibrocystic disease, or abnormal mammograms should be monitored with particular care if they elect to use OC's.

4. **Hepatic Tumors**—Benign hepatic adenomas have been found to be associated with use of OC's. One study showed that OC's with high hormonal potency were associated with higher risk than lower potency OC's. Although benign, hepatic adenomas may rupture and may cause death through intra-abdominal hemorrhage. This has been reported in short-term as well as long-term users. Two studies relate risk with duration of use of OC's, the risk being much greater after 4 or more years' use. While hepatic adenoma is rare, it should be considered in women presenting abdominal pain and tenderness, abdominal mass or shock. A few cases of hepatocellular carcinoma have been reported in women on OC's. Relationship of these drugs to this type of malignancy is not known.

5. **Use in or Immediately Preceding Pregnancy, Birth Defects in Offspring, and Malignancy in Female Offspring**—Use of female sex hormones—both estrogenic and progestational agents—during early pregnancy may seriously damage the offspring. It has been shown that females exposed in utero to diethylstilbestrol, a nonsteroidal estrogen, have increased risk of developing in later life a form of vaginal or cervical cancer, ordinarily extremely rare. This risk has been estimated to be of the order of 1 in 1,000 exposures or less. Although there is no evidence now that OC's further enhance risk of developing this type of malignancy, such patients should be monitored with particular care if they elect to use OC's. Furthermore, 30 to 90% of such exposed women have been found to have epithelial changes of the vagina and cervix. Although these changes are histologically benign, it is not known whether this condition is a precursor of vaginal malignancy. Male children so exposed may develop abnormalities of the urogenital tract. Although similar data are not available with use of other estrogens, it cannot be presumed they would not induce similar changes. An increased risk of congenital anomalies, including heart defects and limb defects, has been reported with use of sex hormones, including OC's, in pregnancy. One case-control study estimated a 4.7-fold increase in risk of limb-reduction defects in infants exposed in utero to sex hormones (OC's, hormonal withdrawal tests for pregnancy, or attempted treatment for threatened abortion). Some exposures involved only a few days. Data suggest that risk of limb-reduction defects in exposed fetuses is somewhat less than 1 in 1,000 live births. In the past, female sex hormones have been used during pregnancy in an attempt to treat threatened or habitual abortion. There is considerable evidence that estrogens are ineffective for these indications, and there is no evidence from well-controlled studies that progestogens are effective. There is some evidence that triploidy and possibly other types of polyploidy are increased among abortuses from women who become pregnant soon after ceasing OC's. Embryos with these anomalies are virtually always aborted spontaneously. Whether there is an overall increase in spontaneous abortion of pregnancies conceived soon after stopping OC's is unknown. It is recommended that, for any patient who has missed 2 consecutive periods, pregnancy should be ruled out before continuing OC's. If the patient has not adhered to the prescribed schedule, the possibility of pregnancy should be considered at time of first missed period, and further use of OC's should be withheld until pregnancy has been ruled out. If pregnancy is confirmed, the patient should be apprised of the potential risks to the fetus, and advisability of continuation of the pregnancy should be discussed. It is also recommended that women who discontinue OC's with intent of becoming pregnant use an alternate form of contraception for a period of time before attempting to conceive. Many clinicians recommend 3 months, although no precise information is available on which to base this. The administration of progestogen-estrogen combinations to induce withdrawal bleeding should not be used as a test of pregnancy.

6. **Gallbladder Disease**—Studies report increased risk of surgically confirmed gallbladder disease in users of OC's and estrogens. In one study, increased risk appeared after 2 years' use and doubled after 4 or 5 years' use. In one of the other studies, increased risk was apparent between 6 and 12 months' use.

7. **Carbohydrate and Lipid Metabolic Effects**—Decrease in glucose tolerance has been observed in a significant percentage of patients on OC's. For this reason, prediabetic and diabetic patients should be carefully observed while on OC's. Increase in triglycerides and total phospholipids has been observed in patients on OC's; clinical significance of this finding remains to be defined.

8. **Elevated Blood Pressure**—Increase in blood pressure has been reported in patients on OC's. In some women, hypertension may occur within a few months of beginning OC's. In the 1st year of use, prevalence of women with hypertension is low in users and may be no higher than that of a comparable group of nonusers. Prevalence in users increases, however, with longer exposure, and in the 5th year of use is 2½ to 3 times the reported prevalence in the 1st year. Age is also strongly correlated with development of hypertension in OC users.

Women who previously have had hypertension during pregnancy may be more likely to develop elevation of blood pressure on OC's. Hypertension that develops as a result of taking OC's usually returns to normal after discontinuing the drug.

9. **Headache**—Onset or exacerbation of migraine or development of headache of a new pattern which is recurrent, persistent, or severe, requires discontinuation of OC's and evaluation of the cause.

10. **Bleeding Irregularities**—Breakthrough bleeding, spotting, and amenorrhea are frequent reasons for patients discontinuing OC's. In breakthrough bleeding, as in all cases of irregular

time or change to another OC may solve the problem. Changing to an OC with a higher estrogen content, while potentially useful in minimizing menstrual irregularity, should be done only if necessary, since this may increase risk of thromboembolic disease. Women with past history of oligomenorrhea or secondary amenorrhea or young women without regular cycles may have a tendency to remain anovulatory or to become amenorrheic after discontinuing OC's. Women with these pre-existing problems should be advised of this possibility and encouraged to use other methods. Post-use anovulation, possibly prolonged, may also occur in women without previous irregularities.

11. **Ectopic Pregnancy**—Ectopic as well as intrauterine pregnancy may occur in contraceptive failures.

12. **Breast-feeding**—OC's given in the postpartum period may interfere with lactation and decrease quantity and quality of breast milk. Furthermore, a small fraction of the hormones in OC's has been identified in the milk of mothers on OC's; effects, if any, on the breast-fed child have not been determined. If feasible, defer OC's until infant has been weaned.

**Precautions—GENERAL**—1. A complete medical and family history should be taken prior to initiation of OC's. Pretreatment and periodic physical examinations should include special reference to blood pressure, breasts, abdomen and pelvic organs, including Pap smear and relevant laboratory tests. As a general rule OC's should not be prescribed for longer than 1 year without another physical examination.

2. Under influence of estrogen-progestogen preparations, pre-existing uterine leiomyomata may increase in size.

3. Patients with history of psychic depression should be carefully observed and the drug discontinued if depression recurs to a serious degree. Patients becoming significantly depressed while on OC's should stop OC's and use an alternate method to try to determine whether the symptom is drug-related.

4. OC's may cause some degree of fluid retention. They should be prescribed with caution, and only with careful monitoring, in patients with conditions which might be aggravated by fluid retention, such as convulsive disorders, migraine syndrome, asthma, or cardiac or renal insufficiency.

5. Patients with a past history of jaundice during pregnancy have an increased risk of recurrence while on OC's. If jaundice develops, OC's should be discontinued.

6. Steroid hormones may be poorly metabolized in patients with impaired liver function and should be administered with caution.

7. OC users may have disturbances in normal tryptophan metabolism which may result in a relative pyridoxine deficiency. Clinical significance is undetermined.

8. Serum folate levels may be depressed by OC's. Since the pregnant woman is predisposed to development of folate deficiency and incidence of folate deficiency increases with increasing gestation, it is possible that if a woman becomes pregnant shortly after stopping OC's, she may have a greater chance of developing folate deficiency and complications attributed to this deficiency.

9. The pathologist should be advised of OC therapy when relevant specimens are submitted.

10. Certain endocrine- and liver-function tests and blood components may be affected by estrogen-containing OC's:

a. Increased sulfobromophthalein retention. b. Increased prothrombin and factors VII, VIII, IX, and X. c. Decreased antithrombin III. d. Increased norepinephrine-induced platelet aggregability. e. Increased thyroid-binding globulin (TBG) leading to increased circulating total-thyroid hormone, as measured by protein-bound iodine (PBI), T4 by column, or T4 by radioimmunoassay. Free T3 resin uptake is decreased, reflecting the elevated TBG; free T4 concentration is unaltered. f. Decreased pregnandiol excretion. g. Reduced response to metyrapone test.

**Information for the Patient**—See Patient Package Labeling. **Drug Interactions**—Reduced efficacy and increased incidence of breakthrough bleeding have been associated with concomitant use of rifampin. A similar association has been suggested with barbiturates, phenylbutazone, phenytoin sodium, ampicillin and tetracycline.

**Carcinogenesis**—See Warnings on carcinogenic potential of OC's.

**Pregnancy**—Category X. See Contraindications, Warnings.

**Nursing Mothers**—See Warnings.

**Adverse Reactions**—An increased risk of these serious adverse reactions has been associated with use of OC's (see Warnings): thrombophlebitis, pulmonary embolism, coronary thrombosis, cerebral thrombosis, cerebral hemorrhage, hypertension, gallbladder disease, benign hepatomas, congenital anomalies. There is evidence of an association between the following conditions and use of OC's although additional confirmatory studies are needed: mesenteric thrombosis, neuro-ocular lesions, e.g., retinal thrombosis and optic neuritis.

The following adverse reactions have been reported in patients on OC's and are believed to be drug-related. Nausea and/or vomiting, usually the most common adverse reactions, occur in approximately 10 percent or less of patients during the first cycle. Other reactions, as a general rule, are seen much less frequently or only occasionally. Gastrointestinal symptoms (such as abdominal cramps and bloating); breakthrough bleeding, spotting, change in menstrual flow; dysmenorrhea, amenorrhea during and after treatment, temporary infertility after discontinuance of treatment; edema; chloasma or melasma which may persist; breast changes: tenderness, enlargement, and secretion; change in weight (increase or decrease); change in cervical erosion and cervical secretion; possible diminution in lactation when given immediately postpartum; cholestatic jaundice; migraine; increase in size of uterine leiomyomata; rash (allergic); mental depression; reduced tolerance to carbohydrates; vaginal candidiasis; change in corneal curvature (steepening); intolerance to contact lenses.

The following adverse reactions have been reported in users of OC's, and the association has been neither confirmed nor refuted: premenstrual-like syndrome, cataracts, changes in libido, chorea, changes in appetite, cystitis-like syndrome, headache, nervousness, dizziness, hirsutism, loss of scalp hair, erythema multiforme, erythema nodosum, hemorrhagic eruption, vaginitis, porphyria.

**Acute Overdose**—Serious ill effects have not been reported following acute ingestion of large doses of OC's by young children. Overdose may cause nausea, and withdrawal bleeding may occur in females.

**LOOVRAL**® each tablet contains  
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# Dilation and evacuation: A preferred method of midtrimester abortion

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A comparison between two methods of therapeutic termination of midtrimester pregnancies was performed. Twenty-nine patients at 13 to 16 weeks' gestation underwent dilation and evacuation (D and E). A second group, of 29 patients at 16 to 19 weeks' gestation underwent therapeutic abortion by prostaglandin  $F_{2\alpha}$  instillation. There was a significantly lower complication rate (6.9% versus 55%) and a shorter hospital stay of 1 full day in the D and E group. Also, the blood loss was not significantly greater in the D and E group. (AM. J. OBSTET. GYNECOL. 139:329, 1981.)

TRADITIONALLY, the gestational age limit for terminating a pregnancy by dilation and evacuation (D and E) has been 12 weeks. The origin of this dictum is uncertain and its basis is elusive, but only recently has it been questioned.<sup>2, 3, 5-9</sup>

The 12-week gestational age limit for D and E creates a number of medical, psychological, and financial problems that could be alleviated if the 12-week "veil" could be withdrawn. For these reasons, our institution decided to perform a limited number of second-trimester abortions on pregnancies found to be at 13 to 16 weeks. They would be compared to a second group of midtrimester therapeutic abortions terminated by prostaglandin  $F_{2\alpha}$  (PGF<sub>2 $\alpha$</sub> ) instillation (TAPI) at 16 to 19 weeks' gestation. This second method is the standard procedure of midtrimester pregnancy termination in our hospital.

## Material and method

Twenty-nine consecutive private patients requesting therapeutic termination of pregnancy were selected during the interval of mid-January, 1979, through April 30, 1980 (Table I). All pregnancies were in the range of 13 to 16 weeks' gestational age. This was de-

termined by date of last menstrual period, clinical examination, and, when necessary, by ultrasound determination. The women ranged in age from 18 to 38 and in parity from 0 to 6.

The second group of 20 patients (Table II) was selected by the medical records department. They were chosen from the 154 private patients who underwent a TAPI during the same time period as the D and E group underwent their procedure. The TAPI group was matched to the D and E group according to age and parity.

Each patient in the study was admitted to the hospital. A history was obtained and physical examination was performed together with routine laboratory tests on the blood and urine. The night prior to D and E, after assessment of uterine size and determination of position, one or two laminaria tents were inserted by means of a sterile technique into the endocervical canal and held in place with loose vaginal packing.

At the beginning of the procedure an intravenous oxytocin infusion was started. The D and E procedures were performed with the patients under general anesthesia. The patient was placed in the supine lithotomy position and, after removal of the packing and laminaria, the vagina was prepared with a povidone-iodine solution. The anterior lip of the cervix was grasped with a Jacob or Teale tenaculum. The endocervical canal was additionally dilated up to a No. 14 Hegar dilator when necessary. A No. 14 curved plastic suction cannula was inserted through the endocervical canal to the lower segment of the uterus and negative pressure of 35 to 65 mm Hg was applied.

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**Table I.** Characteristics of 29 women undergoing D and E

| Characteristic          | Mean ± SD  |
|-------------------------|------------|
| Age (years)             | 25.2 ± 8.0 |
| Gestational age (weeks) | 14.4 ± 0.9 |
| Hospital stay (days)    | 2.0 ± 0.6  |
| Δ Hematocrit (%)        | 4.1 ± 2.5  |

**Table II.** Characteristics of 29 women undergoing TAPI

| Characteristic          | Mean ± SD   |
|-------------------------|-------------|
| Age (years)             | 23.8 ± 7.42 |
| Gestational age (weeks) | 17.3 ± 0.61 |
| Hospital stay (days)    | 3.1 ± 1.5   |
| Δ Hematocrit (%)        | 4.8 ± 2.7   |

**Table III.** Comparison of age and parity: D and E versus TAPI

| Group        | Parity |    | Age (years)<br>(mean ± SD) |
|--------------|--------|----|----------------------------|
|              | 0      | N  |                            |
| D and E      | 18     | 11 | 25.2 ± 8.0                 |
| TAPI         | 14     | 15 | 23.8 ± 7.42                |
| Significance | P = NS |    | P = NS                     |

The chi-square test with the Yates correction was used in statistical analysis. NS: Not significant.

**Table IV.** Comparison of hospital stay: D and E versus TAPI

| Group   | Mean ± SD<br>(days) | Significance |
|---------|---------------------|--------------|
| D and E | 2.00 ± 0.61         | P < 0.01     |
| TAPI    | 3.07 ± 1.46         |              |

Student's t test was used for statistical evaluation.

Amniotic fluid was withdrawn along with fetal and placenta tissue. The suction cannula was sufficient for complete emptying of uterine contents in a small number of cases, usually those at 13 weeks' gestation. Ovum forceps were usually necessary to remove the products of conception brought down by the suction cannula. The sequence of suction and then removal of products of conception by the ovum forceps was repeated until the uterus was emptied. A large, sharp curet was then used to remove any remaining tissue. All procedures were performed by two of the authors (E. L. and E. R.). The patients were transferred to the recovery room when stable and the oxytocin infusion was maintained for 8 hours postoperatively. The next morning, the patient was reassessed and sent home, if free of complications. Postoperative hemoglobin and hematocrit

**Table V.** Comparison of Δ hematocrit\* (percent) for D and E versus TAPI

| Group   | Hematocrit<br>(mean ± SD)<br>(%) | Significance |
|---------|----------------------------------|--------------|
| D and E | 4.07 ± 2.47                      | NS           |
| TAPI    | 4.76 ± 2.72                      |              |

Student's t test was used for statistical evaluation. NS: Not significant.

\*Calculated from values before and after abortion.

**Table VI.** Total complications: D and E versus TAPI

| Complication         | D and E   | TAPI |
|----------------------|-----------|------|
| Cervical laceration  | 2 (5.28*) | 0    |
| Primary curettage†   | 0         | 15   |
| Secondary curettage‡ | 0         | 2    |
| Blood transfusion    | 0         | 2    |
| Fever§               | 1 (27)    | 4    |
| Total                | 3         | 23   |

\*Patient number.

†Primary curettage, within 24 hours of abortion.

‡Secondary curettage, performed 24 hours after abortion.

§Fever was defined as temperature >37.5° C at two consecutive readings, 2 hours apart.

**Table VII.** Comparison of patients with complications: D and E versus TAPI

| Group   | Total No.<br>of patients<br>with complications | Total No.<br>of patients<br>in group | Significance |
|---------|------------------------------------------------|--------------------------------------|--------------|
| D and E | 2                                              | 29                                   | P < 0.001    |
| TAPI    | 16                                             | 29                                   |              |

Statistical analysis was performed by the chi-square test with the Yates correction.

were routinely determined. All patients were seen at follow-up 2 to 3 weeks after abortion.

The TAPI patients received the PGF<sub>2α</sub> injection according to the protocol outlined in the American College of Obstetricians and Gynecologists *Technical Bulletin*, No. 27. In our hospital 2 ml of methylene blue is added to the PGF<sub>2α</sub> injection as a marker for ruptured membranes. An intravenous infusion is started with the injection. If membranes rupture prior to the onset of labor, oxytocin is added to the intravenous infusion to stimulate the onset of contractions. After 24 hours, if there is no cervical dilation or effacement with minimal uterine activity, a second injection of 20 mg PGF<sub>2α</sub> was administered.

When the fetus is expelled, an oxytocin drip is started, if it has not been previously used. Two hours



was the limit allowed for spontaneous expulsion of the placenta. After this time, a speculum examination was performed, and the placenta was removed, if possible. A curettage was performed if the placenta could not be removed. After expulsion of the placenta, the cervix and vagina were routinely examined for lacerations and retained products of conception. The oxytocin drip was continued for 8 hours after abortion and the patient was discharged the next morning, if stable. Follow-up was for 2 to 3 weeks' time.

## Results

**Age and parity.** Table III characterizes the two groups of patients with respect to age and parity. There was no significant difference observed between the groups in either of these parameters.

**Hospital stay.** The mean number of days' stay of each group is shown in Table IV. The 2-day mean stay of the D and E group was significantly less than the 3.07 days in the TAPI group ( $P < 0.01$ ).

**Blood loss.** No significant difference in blood loss was observed in the two groups. Table V shows the mean  $\Delta$  hematocrit of each group, calculated from preabortion and postabortion values.

**Complications.** Table VI shows the complications encountered during this study in both groups. A greater number of total complications was seen in the TAPI group. It should be noted in this table that the same patient may have experienced more than one complication.

A comparison of the number of patients with complications in each group is seen in Table VII. The D and E group had two patients with complications, which is significantly less than the 16 patients from TAPI group ( $P < 0.001$ ).

## Comment

There were early reports in the literature<sup>4, 10</sup> that advocated a 12-week gestational age limit for termination of pregnancy by dilation and suction evacuation. This tenet was not borne out by our experience or by that of others.

Grimes and Cates<sup>8</sup> referred to a "gray zone" from 13 to 16 weeks where masterful inactivity is the rule. We suggest that D and E during this time period is both a safe and preferred method for terminating early midtrimester abortions.

The complication rate in the D and E group was 6.9% (2/29). The complications encountered were two minor cervical lacerations of the anterior lip. These were caused by the tenaculum, not the dilators. Both lacerations were easily repaired with a single No. 2 chromic suture. The use of laminaria tents preoperatively obviated the need to dilate the cervix forcefully. Large cervical lacerations and subsequent cervical incompetence will hopefully be prevented.

The complication rate of the TAPI group was 55% (16/24). This figure includes curettages performed within 24 hours of abortion. In our hospital, a curettage is not a routine postabortion procedure, and thus we include it as a complication of the TAPI procedure.

The complication rate of 6.9% in our D and E group differs significantly from the rates of others.<sup>3, 5-7, 9</sup> This is because we did not distinguish between major and minor complications. In this study, the D and E complication rate is greater because it includes all complications encountered.

In the literature a number of complications of D and E that were not experienced in this study are reported. Postoperative infection, uterine perforation, and retained products of conception have all been reported.<sup>3, 4, 7, 9</sup> These other studies were performed on both early and late midtrimester abortions. Although the size of our D and E group was limited, we suggest that these complications can be minimized by limiting the D and E procedure to the early midtrimester. Also the procedure should be performed by a small number of experienced surgeons.

The hospital stay of the D and E group was 1 full day shorter than that of the TAPI group. This would certainly amount to a significant financial benefit to the patient and involve more efficient use of hospital facilities when compared to the lengthened hospital stay and 50% possibility of a curettage in the TAPI group. A qualitative, but equally important advantage of the D and E method for midtrimester abortion is that it spares the patient the psychological stress associated with a long and painful labor. The anxiety associated with waiting up to 4 weeks until amniocentesis and PGF<sub>2 $\alpha$</sub>  instillation are performed could be avoided as well.

In summary, we feel that D and E should be the method of choice for early midtrimester abortions because of its safety, efficiency, and practicality.

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# Factors influencing morbidity in termination of pregnancy

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This study analyzes the morbidity of therapeutic abortion and identifies predisposing factors. A retrospective case evaluation of 2,105 pregnancy terminations was performed. Major complications occurred in 5% of those undergoing curettage procedures, 41% of women undergoing prostaglandin amnioinfusion, and 17% of those who had hysterotomy as a method of abortion. Age, parity, and history of previous pregnancy termination, had little effect on morbidity. Surgical/technical factors and reliance on amnioinfusion and hysterotomy as procedures of choice for pregnancy termination contributed significantly to the high morbidity rates. This contribution, however, was overshadowed by the influence of patient delay, physician delay, and limited abortion facilities, resulting in 35.4% of pregnancy terminations being performed in the midtrimester. Improvements in abortion technique may lower the incidence of complications, but the need for more appropriate facilities is emphasized. (AM. J. OBSTET. GYNECOL. 139:333, 1981.)

THE HEALTH SCIENCES CENTRE, Winnipeg, serves as the principal center for pregnancy termination in Manitoba, where elective abortion procedures have increased 100% from 1975 to 1980. Paralleling this increase has been a rise in the absolute number of women with abortion-related morbidity, totaling 79% additional in-hospital patient days required for treatment of complications of abortion (1978).

Canadian statistics suggest that morbidity rates associated with abortion in Manitoba are higher than the national average.<sup>1</sup> Further, Manitoba physicians performed a disproportionate number of higher-risk midtrimester procedures. In 1978, for example, 20% of all abortions in Manitoba were done by amnioinfusion and 3% were completed by hysterotomy, com-

pared with Canadian percentages of 7% and 1%, respectively, and United States (in 1977) percentages of only 5.3% and 0.06% for the two procedures.<sup>1, 2</sup>

The present study describes the morbidity of pregnancy termination in this large university hospital and analyzes predisposing factors.

## Material and methods

Eight hundred and ten consecutive first-trimester procedures, performed in 1978, were reviewed for comparison of morbidity rates with midtrimester procedures. A total of 1,295 midtrimester procedures were reviewed, including 1,002 amnioinfusions and 293 hysterotomy/hysterectomy procedures, performed between January 1, 1974, and December 31, 1978.

"Major morbidity" was defined as that which required surgical intervention, medical intervention (such as transfusions, antibiotics, or antiarrhythmic agents), or readmission to the hospital. Other complications constituted minor morbidity. Morbidity rates are expressed as the percentage of patients undergoing a given procedure who developed one or more complications.

## Results

**Patient characteristics.** Forty percent of women studied were teenagers, while 8.7% were over the age of 35. Sixty-eight percent were single, 22% were mar-

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Table I. Interval between initial presentation and abortion procedure

| Weeks' gestation | Initial visit |      | Abortion procedure |      |
|------------------|---------------|------|--------------------|------|
|                  | No.           | %    | No.                | %    |
| <6               | 26            | 1.2  | 1                  | —    |
| 6-8              | 371           | 17.6 | 72                 | 3.4  |
| 8-10             | 485           | 23.0 | 341                | 16.2 |
| 10-12            | 453           | 21.5 | 447                | 21.2 |
| 12-14            | 364           | 17.3 | 249                | 11.8 |
| 14-16            | 236           | 11.2 | 473                | 22.5 |
| 16-18            | 138           | 6.6  | 378                | 18.0 |
| >18              | 29            | 1.4  | 142                | 6.7  |

Table II. Interval between initial assessment by gynecologist to performance of abortion

| Interval in days | No. | %    |
|------------------|-----|------|
| 1-5              | 301 | 14.4 |
| 6-10             | 455 | 21.8 |
| 11-15            | 415 | 19.9 |
| 16-20            | 268 | 12.9 |
| 21-25            | 248 | 11.9 |
| ≥26              | 397 | 19.0 |

ried, and 10% were divorced or separated. Most resided in the urban center of Winnipeg, with 10% coming from isolated northern communities. Primigravid patients represented 52.1% of the study population, while 17.0% had three or more previous pregnancies and 10.2% had undergone previous pregnancy termination.

Table I depicts the duration of the pregnancy from the last normal menstrual period to: (1) the time of presentation to the gynecologist performing the procedure and (2) the time the procedure was actually performed. While 63.3% presented to a gynecologist before the twelfth week of gestation, only 40.8% had the procedure performed during this interval. Delay between points 1 and 2 varied from 1 to 63 days (Table II); the average was 11.8 days for the first-trimester procedure, 19.5 days for amniocentesis, and 16.6 days for hysterotomy. This represents a minimum expression of the delay, as many patients sought medical attention from primary care physicians before attending a gynecologist.

**Abortion techniques.** All pregnancy terminations require approval by a hospital Therapeutic Abortion Committee, in accordance with the Criminal Code of Canada.

Departmental policy limited curettage to a maximum of 10 weeks' gestation in primigravid patients and 11 weeks' gestation in multigravid patients. More than 89% of first-trimester procedures were performed on

Table III. Factors associated with hysterotomy/hysterectomy

| Procedure                               | No. |
|-----------------------------------------|-----|
| <i>Hysterotomy:</i>                     |     |
| Combined with abdominal tubal ligation  | 271 |
| Combined with other abdominal procedure | 2   |
| Secondary—failed curettage              | 5   |
| Secondary—failed amniocentesis          | 3   |
| Other                                   | 3   |
| Total                                   | 284 |
| <i>Hysterectomy:</i>                    |     |
| Carcinoma in situ                       | 4   |
| Leiomyomas                              | 3   |
| "Sterilization"                         | 1   |
| Secondary—hemorrhage after hysterotomy  | 1   |
| Total                                   | 9   |

Table IV. Complications of 810 first-trimester abortions

| Complication                                     | No. |
|--------------------------------------------------|-----|
| <i>Intraoperative:</i>                           |     |
| Cervical laceration—required suturing            | 25  |
| Suspected uterine perforation                    | 2   |
| Proved uterine perforation*                      | 4   |
| Hemorrhage†                                      | 9   |
| Anesthetic complications‡                        | 19  |
| Total                                            | 59  |
| <i>Postoperative:</i>                            |     |
| Parametritis                                     | 2   |
| Endometritis                                     | 9   |
| Uterine bleeding/retained products of conception | 9   |
| Postabortion syndrome§                           | 2   |
| Other                                            | 4   |
| Total                                            | 26  |

\*All had laparoscopy and two had laparotomy following perforation.

†Three patients required a total of eight transfusions.

‡Six required admission for persistent emesis or arrhythmia.

§Postabortion syndrome is characterized by uterine atony and hematometra (see reference 3).

an outpatient basis. Three quarters of those admitted had a tubal ligation concomitant with the abortion. General anesthesia was used in 97.7% of cases. Following cervical dilatation with Hegar dilators, evacuation of the uterus was routinely done with rigid plastic suction curets, followed by sharp curettage.

Prostaglandin F<sub>2α</sub> as a midtrimester abortifacient was administered as a single intra-amniotic infusion of 45 to 55 mg if no adverse reaction had followed a 5 mg test dose. More than one attempt was required to achieve infusion in 18.2% of the 1,002 women, and 2.4% of patients were discharged after repeated attempts failed. Instillation-to-abortion time (IAT) was 19.2 ± 9.2 hours; however, IAT for 47 women (4.7%)



Table V. Complications of 1,002 amniotomies

| Complication                              | No.  |
|-------------------------------------------|------|
| Failed attempt*                           | 24   |
| Failed instillation†                      | 83   |
| Immediate complications:                  |      |
| Hypersensitivity reaction                 |      |
| Mild                                      | 213  |
| Severe                                    | 28‡  |
| Cervical laceration                       | 7‡   |
| IAT >24 hr                                | 57‡  |
| Incomplete abortion§                      | 259‡ |
| "Precautionary"¶                          | 47‡  |
| Hemorrhage                                | 80   |
| (22 required transfusion‡)                |      |
| Febrile morbidity¶                        | 120  |
| Parametritis/endometritis                 | 4‡   |
| Urinary tract infection                   | 2‡   |
| Unspecified infection—treated             | 23‡  |
| Anesthetic complications (306 procedures) | 2    |
| Other (4‡)                                | 14   |
| Total                                     | 838  |
| Delayed complications:                    |      |
| Bleeding                                  | 22   |
| Endometritis/parametritis                 | 24   |
| Other                                     | 15   |
| Total                                     | 61   |

\*No infusion, patient discharged.  
†No labor, required second instillation.  
‡Major complication.  
§Required curettage to complete procedure.  
¶See text.  
¶Temperature >38° C for 12 hours, no antibiotics, no curettage.

was >48 hours. Abortion was complete in 306 patients (30.6%). Manual removal of the placenta was required in 41.8%, and curettage for completion was done in 27%. Mean duration of the third stage of labor was 3.4 ± 4.3 hours. All patients were admitted to the hospital for the entire procedure with a mean hospital stay of 2.9 ± 1.1 days.

At our institution, hysterotomy was used as a primary procedure for abortion, especially when sterilization was also requested (Table III). This procedure resulted in a hospital stay of 7.4 ± 3.1 days.

**Abortion-related morbidity.** There were no abortion-related deaths in the 5 years under review. The complications of the three procedures employed are presented in Tables IV to VI.

With suction curettage, the rate of cervical laceration requiring suturing was 3.09 per 100 abortions, while the transfusion rate was 0.37 per 100 abortions. Sixteen women with postoperative complications required repeat curettage, while two underwent hysterotomy. Patient morbidity rates for this procedure were 2.1% minor morbidity and 5.2% major morbidity, for an overall rate of 7.25%.

In the amniotomies group, the main sources of

Table VI. Complications of 293 hysterotomy/hysterectomy procedures

| Complication                  | No. |
|-------------------------------|-----|
| Anesthetic* (297 procedures)  | 8   |
| Hemorrhage†                   | 5   |
| Reoperation                   | 4‡  |
| Endometritis/parametritis     | 4‡  |
| Febrile morbidity             | 88  |
| Urinary tract infection       | 16‡ |
| Wound infection               | 6‡  |
| Unspecified infection—treated | 26‡ |
| Other (7‡)                    | 27  |

\*Two patients required antiarrhythmic agents (major complication).  
†Three patients required a total of eight transfusions (major complication).  
‡Major complication.

Table VII. Complication rates by procedure

| Procedure             | Complications |         |
|-----------------------|---------------|---------|
|                       | Total         | Major   |
| First-trimester       | 7.2%*         | 5.2%†   |
| Midtrimester (total): | 59.2%*        | 36.6%†  |
| Amniotomies           | (61.4%)       | (42.1%) |
| Hysterotomy           | (51.5%)       | (17.7%) |

\*Chi-square test = 563.24, p<0.0001, when total complication rates between first-trimester and midtrimester procedures are compared.  
†Chi-square test = 236.05, p<0.001 when major complication rates are compared.

minor morbidity were mild hypersensitivity reactions (in 21.3%) and febrile morbidity (in 10.2%), while the principal sources of major morbidity related to retained products of conception. Three hundred forty-two women (34.1%) required curettage. In 47, curettage performed on clinical grounds yielded no retained products ("precautionary" curettage). Thirty-six patients underwent curettage for complications arising after discharge from the hospital.

Women undergoing amniotomies had an incidence of 19.4% minor morbidity and 42.1% major morbidity, yielding an overall rate of 61.5%.

The majority of minor morbidity with hysterotomy related to fever, while most major morbidity was due to sepsis, with three patients requiring reoperation for severe postoperative hemorrhage. The morbidity rates associated with this procedure were 33.8% minor morbidity and 17.7% major morbidity, 51.5% overall.

**Factors related to morbidity.**

**Age.** Women less than 20 years old had a 34% increased risk of prostaglandin hypersensitivity compared to those 20 and older. This was the only indi-

vidual complication for which risk was elevated on the basis of lower age. However, analysis of the entire study group revealed that teenagers have a 35% greater chance of incurring at least one complication of abortion. This is associated with the fact that teenagers presented to their gynecologists an average of 8.5 days later in pregnancy than older women. While they compose 40% of the study population, teenagers were disproportionately divided between first-trimester (only 34% less than 20 years old) and midtrimester (56% less than 20 years old) procedures. This disproportion is highly statistically significant ( $t = 8.81$ ,  $p < 0.001$ ).

**Parity.** Nulliparous patients, compared with women who were para 1 or greater, did not have a markedly increased risk of complication. While nulliparous patients as a group had an increased risk of hypersensitivity to prostaglandin (risk factor 1.45), they had no significant difference in IAT with amnioinfusion, nor did they differ significantly in the rate of cervical laceration at curettage. In fact, they had a reduced risk of retained placenta after amnioinfusion, which was only 0.79 of the risk of parous patients. Nulliparous patients sought abortion an average of 6.1 days later, but this did not influence their distribution between first- and second-trimester procedures, as they formed 58% of each group.

**Gestational age.** In first-trimester procedures, a definite demarcation of morbidity rate exists at gestations of  $>10$  weeks' duration. Patients beyond this time had an increase of 81% in morbidity, compared with those  $<10$  weeks. In the midtrimester procedures, no such critical point of gestational length exists, as 73% of all procedures were done in a narrow gestational range from  $14\frac{1}{2}$  to 16 weeks.

Gestational duration at time of presentation to a gynecologist did not correlate with any differences in morbidity within a given procedure group. It also does not necessarily determine choice of procedure. In fact, 242 women who underwent amnioinfusion and 138 who had hysterotomy (a total of 29.3% of midtrimester abortions) were seen initially at a gestational age conforming to the existing guidelines for suction curettage.

**Social factors.** Geographic isolation, while associated with delay in reporting to a gynecologist, did not confer an added risk of complication. Marital status did not correlate with any significant difference in morbidity or with any differences in distribution between first-trimester and midtrimester procedures.

**Surgical/technical factors.** In the first trimester, the addition of tubal ligation, by any approach, to the curettage produced no significant additional morbidity.

In amnioinfusion procedures, the principal variable was the time the attending physician was willing to wait before actively removing the placenta. Those patients in whom the placenta was retained more than 4 hours had 32% more immediate complications. However, those in whom abortion was completed within 4 hours had almost twice the risk of delayed complications after discharge.

Several aspects of abortion technique, such as use of laminaria, variations in instruments, and alternate routes of prostaglandin administration, differ from those reported in other large studies but were not variables within our study population.

The variable most significantly associated with abortion-related morbidity was choice of procedure. Patients undergoing midtrimester procedures had seven times the risk of major complications compared to those having first-trimester procedures (Table VII). The choice of procedure was not absolutely dictated by the gestational age (see above). Patients were delayed, apparently by nonmedical factors, from the first-trimester to the midtrimester. While patients waited an average of 19.5 days for amnioinfusion and 16.6 days for hysterotomy, those who presented to their gynecologists at a gestation conforming to the guidelines for curettage (but subsequently underwent midtrimester abortion) waited an average of 29.6 days for the procedure.

### Comment

As a tertiary care center, our institution may be expected to have higher morbidity rates because of referral of higher-risk patients for higher-risk procedures. For example, all midtrimester abortions performed in Manitoba are done at our hospital. This study, however, identifies several other factors contributing to high morbidity.

Age and parity appear directly to contribute little to morbidity. Young patients, however, seek care later in pregnancy and are thus more likely to undergo abortion late in the first trimester or in the midtrimester with the attendant morbidity.

This study confirms the relationship between advancing gestation and increasing morbidity observed by Cates and associates.<sup>4</sup> Over 50% of curettages were performed at  $>10$  weeks' gestation, with increased frequencies of incomplete curettage and postabortion infection compared to those of other studies.<sup>5</sup>

The rates of cervical laceration and transfusion are much higher than those found by Grimes and Cates<sup>6</sup> for curettage at  $<12$  weeks' gestation. This increase is likely related to the difficulty of cervical dilatation with



Hegar dilators.<sup>7</sup> The use of laminaria as reported<sup>8</sup> and tapered dilators on a routine basis has since begun in our institution.

Our current use of prostaglandin  $F_{2\alpha}$  differs from that reported elsewhere.<sup>9, 10</sup> While IAT did not differ significantly from that in other reports,<sup>11</sup> the complication rate was higher, suggesting that the routine use of curettage to complete amniotomies<sup>12</sup> might assist in reducing morbidity.

Hysterotomy as a primary procedure for pregnancy termination has an inappropriately high rate of morbidity. It is not a suitable treatment alternative for patients requesting abortion and sterilization and has been abandoned at our hospital.

The current morbidity of abortion in the 11- to 14-week range might be further reduced by introduction of dilatation and evacuation (D and E) procedures, as discussed by several authors.<sup>13-15</sup>

The potential benefits of all these improvements in abortion technique are overshadowed by nonmedical factors that influence the number of midtrimester procedures. During the 5 years studied, 35.4% of all abortions were performed in the midtrimester. Approxi-

mately 70% of women who underwent midtrimester procedures presented after 12 weeks' gestation. This late presenting appears to be a result of both patient and physician delay. Conversely, almost 30% who had midtrimester abortions had presented at gestational ages when prompt provision of curettage would be expected to lower dramatically the risk of morbidity.

The ability to provide such low-risk procedures is governed by the availability of facilities. Only eight of the 77 accredited hospitals in Manitoba have therapeutic abortion committees, and our institution alone accounts for 67% to 78% of abortions performed yearly. For a population of just over one million, current facilities are inadequate.

The morbidity rates for abortion demonstrated by this study are unacceptably high. While improvements in technique may lower the incidence of some complications, the main focus for reduction of morbidity must be the prompt provision of low-risk pregnancy termination procedures, preferably in the first trimester. This awaits the combined efforts of all concerned, including patients, physicians, hospital administrations, and government.

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# Loss of diurnal rhythm in plasma estrone, estradiol, and estriol in women treated with synthetic glucocorticoids at 34 to 35 weeks' gestation

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In order to examine the effect of endogenous rhythms in fetal and/or maternal adrenal activity on diurnal changes in plasma estrone ( $E_1$ ), estradiol ( $E_2$ ), estriol ( $E_3$ ), and progesterone (P) in women at 34 to 35 weeks' gestation, we measured these steroids in a group of six women in whom the endogenous circadian rhythm in cortisol was absent or had been suppressed by treatment with exogenous synthetic glucocorticoids. All steroids were suppressed, and no diurnal rhythms in  $E_1$ ,  $E_2$ ,  $E_3$ , or P were found. Suppression of cortisol and estrogens was greater after dexamethasone (Dx) than after a glucocorticoid-equivalent dosage of prednisone (Pd). Loss of the maternal estrogen rhythms seen in normal patients is consistent with the suggestion that such rhythms may be attributable to diurnal changes in maternal and/or fetal adrenal output of potential precursors. The differences in cortisol and estrogen concentrations after Dx and Pd may indicate dissociation of the glucocorticoid (hyperglycemic) and adrenocorticotrophic hormone-suppressing activities of these compounds. (AM. J. OBSTET. GYNECOL. 139:338, 1981.)

WE HAVE RECENTLY REPORTED that the concentrations of cortisol and unconjugated estriol ( $E_3$ ) in the blood of pregnant women at 30 to 35 weeks' gestation show significant diurnal changes and that the steroids have an inverse relationship to each other.<sup>1, 2</sup> The rhythm of  $E_3$  is different from that of estrone ( $E_1$ ) and estradiol ( $E_2$ ). These results were consistent with the hypothesis that during late pregnancy in the primate

the mother may have an influence on the level of pituitary-adrenal function in the fetus<sup>3</sup> through that maternally derived glucocorticoid which passes into the fetal compartment.<sup>4</sup> To assess further the possible influence of maternal and fetal adrenal precursor production on estrogen rhythms in maternal plasma, we have measured the concentrations of  $E_1$ ,  $E_2$ , and  $E_3$  in women treated with synthetic glucocorticoids at 34 to 35 weeks of gestation. We administered prednisone and dexamethasone because some<sup>5</sup> although not all<sup>6</sup> reports have suggested that the fluorinated glucocorticoids may be less well metabolized in the placenta and might pass more readily into the fetal compartment. We reasoned that if rhythms in maternal estrogen concentrations resulted from diurnal changes in maternal and/or fetal adrenocortical activity, they would be suppressed by the administration of exogenous glucocorticoids, whereas if the rhythms were dependent on factors such as diurnal changes in uterine blood flow,<sup>7</sup> they might not be influenced by exogenous glucocorticoids.

In human pregnancy the major precursor of placental progesterone production is likely to be cholesterol, derived from the maternal circulation.<sup>8</sup> The possibility remains, however, that pregnenolone sulfate from the

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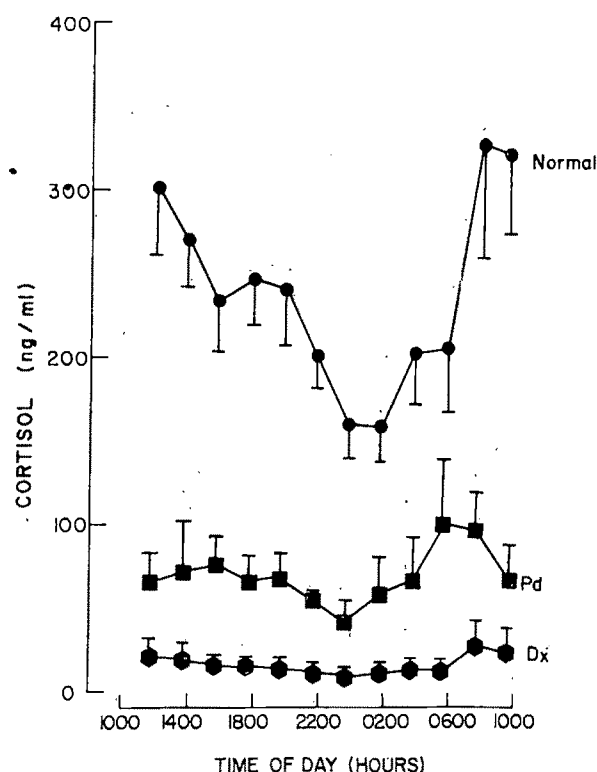


**Fig. 1.** Concentration of cortisol throughout a 24-hour study period in a group of six women at 34 to 35 weeks' gestation treated with either prednisone (Pd, ■—■) or dexamethasone (Dx, ●—●). The concentration of cortisol in the plasma of 11 normal women at 34 to 35 weeks' gestation is shown for comparison (●—●).

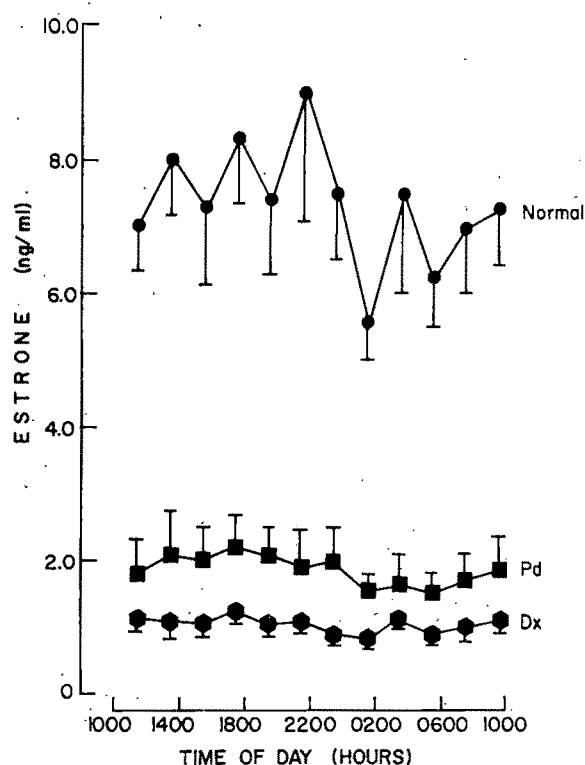


**Fig. 2.** Concentration of  $E_1$  throughout a 24-hour study period in a group of six women at 34 to 35 weeks' gestation treated with either prednisone (Pd, ■—■) or dexamethasone (Dx, ●—●). The concentration of  $E_1$  in the plasma of 11 normal women at 34 to 35 weeks' gestation is shown for comparison (●—●).

fetal and/or maternal adrenal glands could also be utilized for progesterone biosynthesis.<sup>9</sup> Therefore, we compared the concentration of progesterone in the plasma of those patients receiving exogenous glucocorticoids with that in a group of normal women at a comparable stage of gestation.

#### Material and methods

**Patients.** Six women who had received prednisone (10 to 20 mg/day) for medical indications throughout pregnancy were studied for two 24-hour intervals. Details of the patients and the medications used have been described elsewhere.<sup>10</sup> At 34 to 35 weeks of pregnancy, the subjects were hospitalized and a 24-hour (0800 to 0800 hours) study of fetal breathing movements and gross fetal body movements was performed.<sup>10</sup> During the study period, 13 samples of maternal peripheral venous blood (5 ml) were collected at 2-hour intervals from an intravenous catheter that had been introduced into a maternal arm vein. After the first 24-hour study, the patients were sent home. During the following week, prednisone was discontinued and dexametha-

sone was given in equivalent dosage<sup>10</sup> for 4 days. On the fourth day of dexamethasone therapy a second 24-hour study was performed from 0800 to 0800 hours the following day. Both studies were performed with patients at rest in bed. Standardized 800 kcal meals were given at 0800, 1200, and 1700 hours.<sup>10</sup> Cigarette smoking was not permitted during the study.

**Hormone analyses.** The blood samples were collected into heparinized syringes and centrifuged immediately at 4° C; aliquots of the plasma were frozen at -20° C for subsequent analysis by radioimmunoassay (RIA).

Estrone, estradiol, and estriol were measured by RIA with the use of procedures and antibodies which have been described elsewhere.<sup>11</sup> The intra-assay and interassay coefficients of variation varied between 6.2% and 8.7% and 11.2% and 12.8% respectively.

Cortisol was measured by RIA with the use of an antibody that we have described previously.<sup>1, 3</sup> For RIA, [<sup>3</sup>H]-cortisol (specific activity 80 to 100 Ci/mmol, New England Nuclear Ltd.) was purified by thin-layer chromatography in the solvent system chloroform:

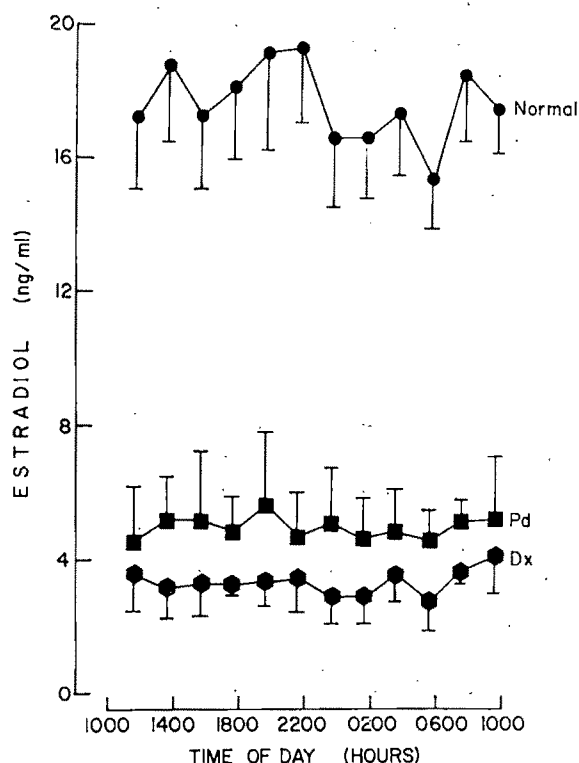


Fig. 3. Concentration of  $E_2$  throughout a 24-hour study period in a group of six women at 34 to 35 weeks' gestation treated with either prednisone (Pd, ■—■) or dexamethasone (Dx, ●—●). The concentration of  $E_2$  in the plasma of 11 normal women at 34 to 35 weeks' gestation is shown for comparison (●—●).

ethanol (95:5, volume:volume). Then 2,000 dpm of [ $^3H$ ]-cortisol was added in 25  $\mu$ l of phosphate-buffered saline (PBS) to the samples of plasma (0.1 ml). Plasma proteins were precipitated with redistilled ethyl alcohol (0.5 ml), and 0.4 ml of the alcohol phase was removed and dried. The sample was chromatographed on microcolumns of LH-20 swollen in methylene chloride:ethanol (98:2), and the eluate containing cortisol was dried and suspended in PBS (1 ml). One aliquot (0.3 ml) was taken for the determination of methodologic losses, and two further aliquots (0.4 and 0.2 ml) were taken for the cortisol RIA. Samples were analyzed in a single assay. The intra-assay variation, calculated as the standard deviation between the duplicate aliquots of individual samples was 11.25%. All results are presented after correction for methodologic losses.

Progesterone was determined by RIA with the use of an antibody and procedures that have been described elsewhere.<sup>11</sup> The intra-assay variation, calculated as the standard deviation between duplicate aliquots of individual samples, was 4.9%. Coelution on micro-Celite columns of authentic radioactive progesterone with

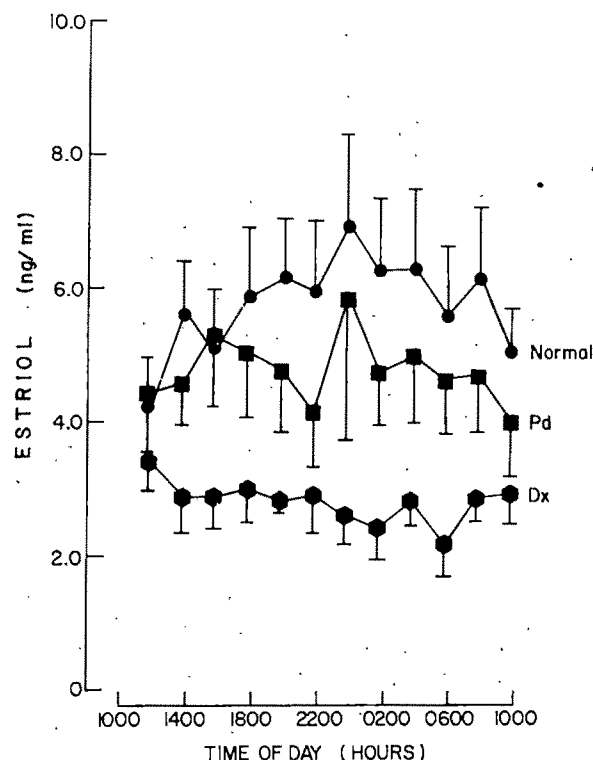


Fig. 4. Concentration of  $E_3$  throughout a 24-hour study period in a group of six women at 34 to 35 weeks' gestation treated with either prednisone (Pd, ■—■) or dexamethasone (Dx, ●—●). The concentration of  $E_3$  in the plasma of 11 normal women at 34 to 35 weeks' gestation is shown for comparison (●—●).

nonradioactive progesterone in samples of plasma from patients treated with either prednisone or dexamethasone was used to confirm the identity of the steroid.

**Analysis of results.** The effects of time on steroid hormone concentrations were assessed by one-way analysis of variance and by Duncan's multiple range analysis. Comparison of mean daily hormone concentrations between different treatment groups was made by means of analyses of variance.

## Results

The outcome of pregnancy in all six patients was normal.<sup>10</sup> The concentrations of steroid hormones in the plasma of these patients have been compared to the steroid concentrations in the plasma of a group of normal (untreated) women at 34 to 35 weeks' gestation.<sup>1</sup>

The mean daily concentration of cortisol was  $17.3 \pm 2.2$  (SEM) ng/ml after dexamethasone and  $67.4 \pm 5.1$  ng/ml after prednisone (Fig. 1). These values respectively represent 95.6% and 75.3% suppression of the



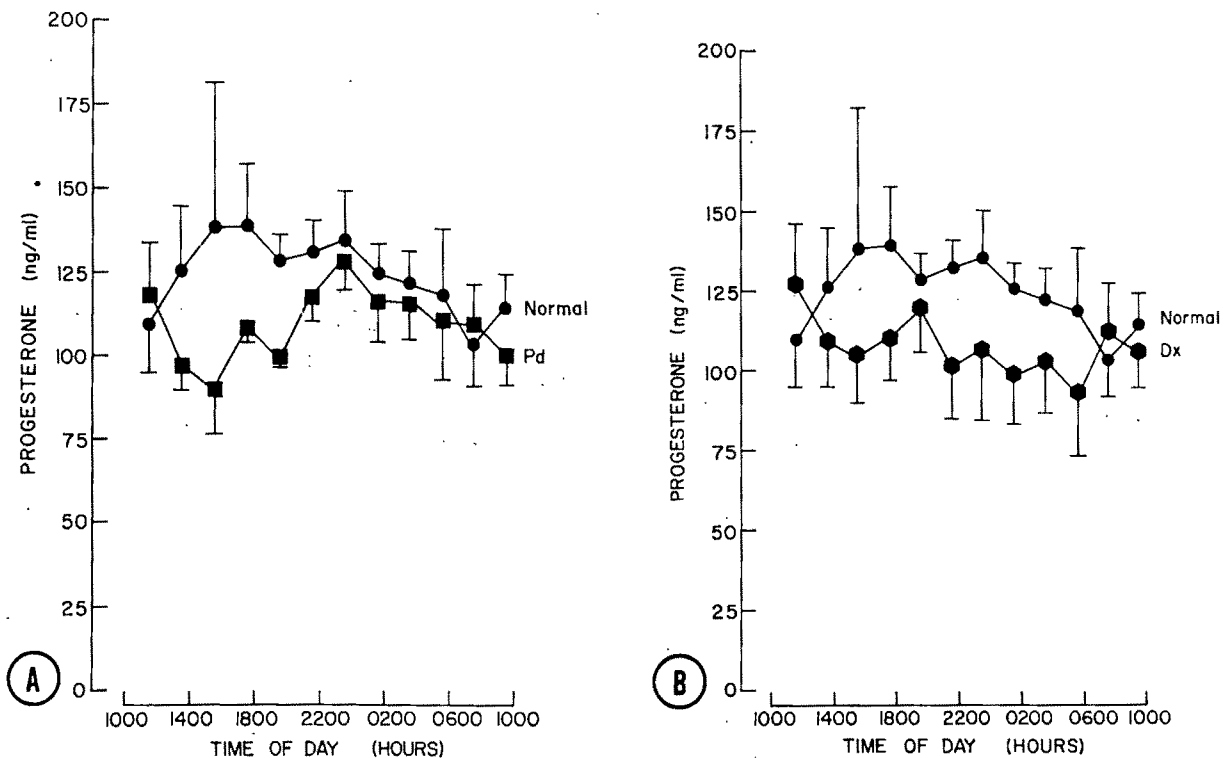


**Fig. 5.** Concentration of progesterone throughout a 24-hour study period in a group of six women at 34 to 35 weeks' gestation treated with either (A) prednisone (Pd, ■—■) or (B) dexamethasone (Dx, ●—●). Comparison is made with the concentration of progesterone in the plasma of 11 normal women at 34 to 35 weeks' gestation (●—●).

mean daily cortisol concentration when compared to values in a group of untreated patients at 34 to 35 weeks' gestation. The concentration of cortisol in the dexamethasone-treated group was significantly lower than in the prednisone-treated patients ( $F_{1, 11} = 141.6$ ,  $P < 0.001$ ). Although the average concentrations of cortisol tended to be higher in the early hours of the morning, especially in the prednisone-treated patients, there was no effect of time on the cortisol concentration in either group ( $P > 0.05$ ).

The diurnal changes in  $E_1$ ,  $E_2$ , and  $E_3$  found in untreated patients at 34 to 35 weeks' gestation were not present in patients treated with prednisone or dexamethasone (time effects: all  $P > 0.05$ ). The mean daily concentrations of  $E_1$ ,  $E_2$ , and  $E_3$  and the percentage of suppression compared to values in untreated patients were  $1.04 \pm 0.4$  ng/ml (86% suppression),  $3.37 \pm 0.09$  ng/ml (81%), and  $2.83 \pm 0.09$  ng/ml (53%), respectively, after dexamethasone and  $1.89 \pm 0.07$  ng/ml (74%),  $5.02 \pm 0.09$  ng/ml (71%), and  $5.04 \pm 0.13$  ng/ml (16%) after prednisone (Figs. 2 to 4). The daily mean concentration of each estrogen was significantly lower after dexamethasone than after prednisone (ANOVAR, all  $P < 0.01$ ).

The mean daily progesterone concentration in six untreated women at 34 to 35 weeks of gestation was  $125.1 \pm 3.2$  ng/ml. Analysis of variance revealed a significant effect of both dexamethasone and prednisone on progesterone concentrations (treatment  $F_{2, 22} = 5.67$ ,  $P < 0.05$ , both treatments  $P < 0.05$  by least-squares difference). The mean daily concentrations of progesterone were  $100.7 \pm 8.5$  and  $110.5 \pm 3.2$  ng/ml after dexamethasone and prednisone, respectively. There was no significant difference between the two treatment groups (Fig. 5).

#### Comment

Previously, we have reported diurnal changes in the concentrations of cortisol,  $E_1$ ,  $E_2$ , and  $E_3$  in women at 34 to 35 weeks of pregnancy.<sup>1</sup> The rhythm in estrone followed that in cortisol with a lag-time of about 2 hours and could have reflected changes in the secretion of  $C_{19}$  estrogen precursor steroids by the maternal adrenal glands.<sup>12</sup> Conversely the rhythm in plasma  $E_3$  showed an inverse pattern to that of cortisol.<sup>1, 2</sup> This might be explained by diurnal changes in the production of  $\Delta^5 C_{19}$  steroids by the fetal adrenal gland which in turn could be regulated in a tonic fashion by mater-

nally derived glucocorticoid.<sup>1</sup> The present study shows, in women treated with prednisone and dexamethasone at 34 to 35 weeks of gestation, that the mean concentrations of cortisol,  $E_1$ ,  $E_2$ , and  $E_3$  in maternal plasma are reduced and the diurnal changes in the plasma levels of these hormones are abolished.

It is likely that suppression of maternal adrenal steroidogenesis contributes to the fall in plasma  $E_1$  and  $E_2$ . In addition, approximately 50% of maternal  $E_2$  is derived from precursors of fetal adrenal origin.<sup>1</sup> Transplacental passage of the exogenous glucocorticoid to the fetus, with subsequent suppression of fetal pituitary-adrenal function, could, therefore, account for some of the change in  $E_2$ . However maternal unconjugated  $E_3$  may be a better index of fetal adrenal steroid output than  $E_2$ . After suppression of the endogenous maternal cortisol rhythm, by replacement with equivalent amounts of exogenous glucocorticoid, the diurnal rhythm in maternal  $E_3$  was also abolished, even though the percentage suppression in  $E_3$  was only 15% to 20%, as in the prednisone group. The difference in the degree to which  $E_3$  was suppressed after prednisone or dexamethasone could result from differences in the extent to which these steroids are metabolized in the placenta<sup>5</sup> and by limited  $11\beta$ -hydroxylation of prednisone in the fetus.<sup>14</sup> The relative amounts of prednisone and dexamethasone used in this study are generally accepted to provide glucocorticoid equivalents and plasma glucose concentrations were similar in both groups of patients.<sup>10</sup> The differential effect on maternal cortisol may be attributable to multiple binding sites for glucocorticoids in brain tissue and the binding of prednisone and dexamethasone to different receptor populations.<sup>15</sup> A dissociation be-

tween adrenocorticotrophic hormone-suppressing, eosinopenic, and hyperglycemic activities on cortisol analogues has been reported previously.<sup>16</sup>

The concentration of progesterone in maternal plasma was also lower in women treated with exogenous glucocorticoids than in normal patients at 34 to 35 weeks' gestation. This result would be consistent with the possibility that the fetal adrenal secretes pregnenolone sulfate,<sup>9</sup> which could be utilized for placental progesterone production. However, we cannot exclude the possibility that the decrease in progesterone (as well as in the estrogens) is influenced by changes in uterine blood flow or by alterations in metabolic clearance rate consequent upon glucocorticoid administration. Because all six patients were delivered of healthy infants at term, with normal birth weights,<sup>10</sup> any effect on uterine and placental blood flow seems likely to be of limited importance.

Suppression of urinary  $E_3$  and plasma  $E_1$  and  $E_2$  after exogenous glucocorticoids is well documented.<sup>4</sup> The present study has shown that, in addition to the decrease in concentration of these steroids after exogenous glucocorticoids, the diurnal pattern in their plasma concentration is lost, as is that of maternal cortisol, an index of maternal adrenal steroid output. These results would support the suggestion that diurnal rhythms in maternal estrogen concentrations could result from rhythms in the provision of precursors from the maternal and/or fetal adrenal glands.

We wish to acknowledge the active interest of Mrs. Karen Campbell and Miss Lesley Carmichael in the conduct of these experiments.

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#### **Problems of the Aging Female**

The University of Texas Health Science Center at Houston, Medical School, Department of Obstetrics and Gynecology and Division of Continuing Education, will present a course, "Problems of the Aging Female," Friday, February 13, 1981, at Stouffer's Greenway Plaza Hotel, Six Greenway Plaza East, Houston, Texas.

For information write: The University of Texas, Health Science Center at Houston, Division of Continuing Education, P. O. Box 20367, Houston, Texas 77025.

#### **Diagnostic Ultrasound Update 1981**

A program, entitled "Diagnostic Ultrasound Update 1981," to be held February 11-14, 1981, in San Juan, Puerto Rico, is being organized and coordinated by the Division of Diagnostic Ultrasound of Thomas Jefferson University.

For information please contact either Barry B. Goldberg, M.D., Director, or Kathy Bonner, Education Secretary, Thomas Jefferson University Hospital, Division of Diagnostic Ultrasound, Curtis Building, Fifth Floor, 1015 Walnut St., Philadelphia, Pennsylvania 19107.

#### **Clinical Focus—Obstetrics and Gynecology**

The SUNY-Upstate Medical Center announces a program, "Clinical Focus: Obstetrics & Gynecology," to be held February 15-21, 1981, at the Hilton Inn, Lake Placid, New York.

For more information contact: Program Coordinator, Office of Continuing Education, Upstate Medical Center, State University of New York, 750 East Adams St., Syracuse, New York 13210.

# Effects of external physical stimulation on fetuses near term

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Measurements were made in 17 healthy pregnant women between 36 and 42 weeks' gestational age 90 minutes before and after a standard physical stimulus. The mean fetal heart rate was  $138 \pm 0.4$  bpm; the median value of the 5-minute standard deviation of successive heartbeat intervals was 30.3 msec, and the percentage of time spent making gross fetal body movements was  $11.1\% \pm 0.4\%$ . These measurements did not change following stimulation of fetuses. Fetal breathing movements diminished over the 180-minute observation period but did not appear to be changed by fetal stimulation. During studies, periodic increases separated by periodic decreases in fetal heart rate, fetal heart rate variability, gross fetal body movements, and fetal breathing movements were measured, and these changes did not seem to be related to external stimulation of fetuses. Periodic changes in these measurements may have been related to underlying patterns of fetal behavior but were not usually influenced by external physical stimulation of fetuses. (AM. J. OBSTET. GYNECOL. 139:344, 1981.)

ANTEPARTUM FETAL HEART rate recordings,<sup>1-3</sup> measurements of fetal movements,<sup>4, 5</sup> and fetal breathing activity<sup>6</sup> have been suggested as methods for assessing fetal health. Absence of fetal movements alone or in association with lack of fetal heart rate variability has been suggested as a sign of fetal ill health. However, healthy fetuses and healthy newborn infants also normally exhibit patterns of decreased heart rate variability and patterns of decreased or absent gross fetal body movements for prolonged periods of time.<sup>7-11</sup> Therefore, it is necessary to account for physiologic patterns

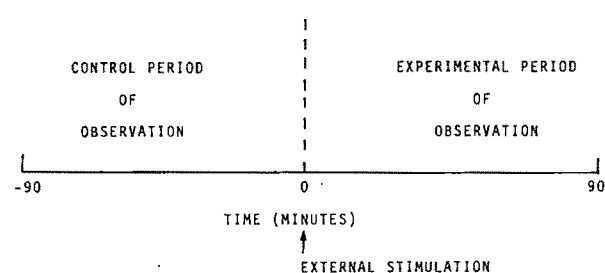


Fig. 1. Experimental design.

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*Presented at the Thirty-sixth Annual Meeting of The Society of Obstetricians and Gynaecologists of Canada, Jasper, Alberta, Canada, June 10-14, 1980.*

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of fetal inactivity before lack of activity and lack of heart rate variability are considered as a sign of fetal ill health.

It was reported that external physical stimulation of the maternal abdomen during episodes of inactivity aroused fetuses into more active patterns of movement.<sup>2, 3</sup> These investigators reasoned that, unlike healthy fetuses, sick fetuses might not be aroused by external stimulation and that external stimulation could be used to separate healthy fetuses monitored during quiet periods from sick fetuses. The purpose of this study was to define, in a group of healthy fetuses, any changes in fetal heart rate, fetal heart rate variability, gross fetal body movement, or fetal breathing



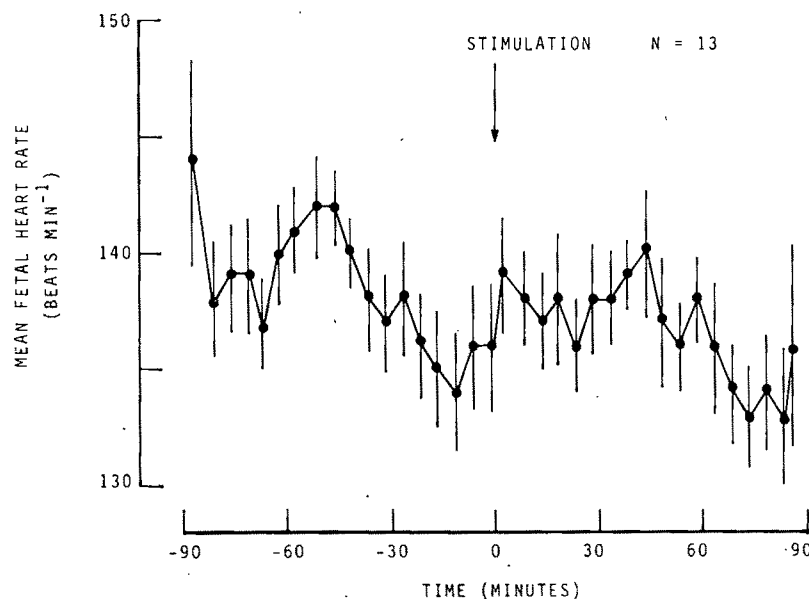


Fig. 2. The mean fetal heart rate ( $\pm$ SEM) was plotted in successive 5-minute intervals in 13 fetuses 90 minutes before and following external physical stimulation.

movements following a standardized external physical stimulation of the maternal abdomen.

#### Methods

**Patients.** Informed consent was obtained from 17 pregnant women between 36 and 42 weeks' gestational age (mean 39 weeks). Fifteen patients were considered in good health and two patients had developed mild pre-eclampsia. Fetal outcome confirmed gestational age and the good health of fetuses studied. The average weight of fetuses at delivery was 3,805 gm (range 3,025 to 4,440 gm). The average Apgar score was 8.8 at 1 minute and 9.2 at 5 minutes. No Apgar score was less than 8 at 1 minute or 9 at 5 minutes. Umbilical cord blood gas values were determined at delivery in 10 of the fetuses. The average umbilical arterial  $P_{O_2}$  was 20.6 mm Hg,  $P_{CO_2}$  was 44.4 mm Hg, and pH was 7.25. There was no umbilical cord arterial pH less than 7.17. Four patients were delivered during labor by cesarean section for cephalopelvic disproportion and one patient had a repeat cesarean section for cephalopelvic disproportion. None of the 17 patients developed fetal distress in labor. All fetuses were in a vertex presentation at the time of the study and at delivery.

**Experimental design.** Patients were studied continuously for 3 hours and studies began between 0900 and 1000 hours. External physical stimulation was performed over a 30-second period by the same investigator in all patients and consisted of 10 vigorous shaking movements of the fundal portion of the uterus fol-

lowed by 10 rocking movements of the fetal head suprapubically. In 14 patients, external physical stimulation of the fetus was performed after a 90-minute control period of observation (Fig. 1). In three other patients, external physical stimulation of the fetus was performed every 15 minutes over a 3-hour study period.

Patients were instructed to fast from midnight the day before the study and were not given any meals during the study period. Maternal peripheral venous blood was sampled for glucose concentrations at the beginning of each study and the mean value for all patients was  $83.3 \pm 3.5$  mg/100 ml. Studies were conducted in a quiet room with patients either sitting upright in bed or resting in a lateral recumbent position. Smoking by patients or observers was not permitted during the study.

**Measurement of fetal heart rate, fetal heart rate variability, gross fetal body movements, and fetal breathing movements.** A mixed maternal and fetal electrocardiogram was obtained from three platinum needle electrodes\* placed subcutaneously in the anterior maternal abdominal wall. Fetal QRS complexes were recognized by an abdominal electrocardiogram processor.† The time between successive fetal QRS complexes, recognized by the processor, was measured on line to within 100  $\mu$ sec by means of a PDP 11 com-

\*Grass Instrument Company, Quincy, Massachusetts.

†Model 15174A, Hewlett-Packard Corp., West Germany.

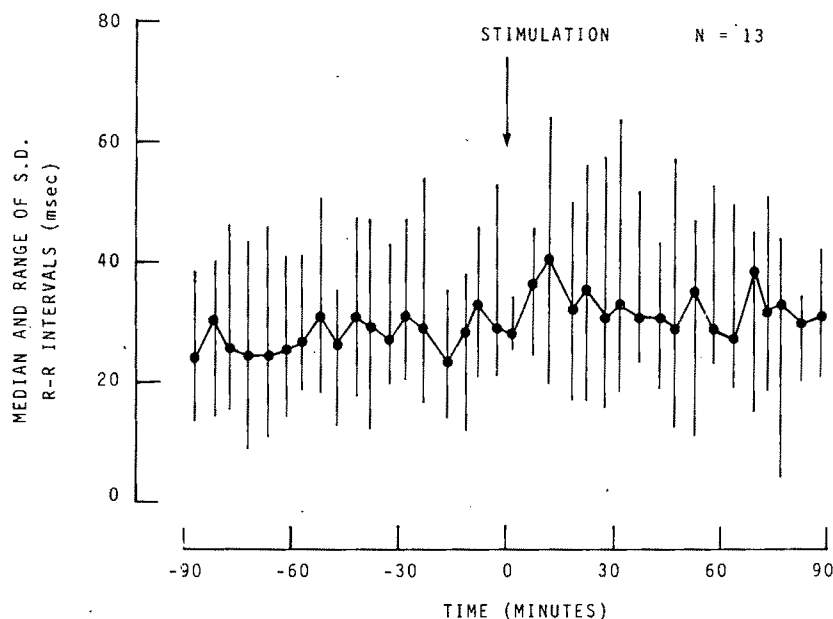


Fig. 3. The median value for the 5-minute SD of successive fetal heartbeat intervals ( $\pm$ range) was plotted in successive 5-minute intervals in 13 fetuses 90 minutes before and following external physical stimulation.

puter. Successive fetal heartbeat intervals were examined by an error rejection routine, similar to that of Dalton and associates,<sup>12</sup> to detect artifacts arising from interference in the signal. The total rejected time was stored and was 15.2% on average (range 7.0% to 29.2%).

The mean fetal heart rate and the standard deviation of successive heartbeat intervals (5-minute SD) were determined every 5 minutes during the 3-hour observation periods. The 5-minute SD of heartbeat intervals has been described as a good measurement of long-term fetal heart rate variability.<sup>12</sup>

Fetal breathing movements and gross fetal body movements were measured with the use of an ADR real-time scanner\* as previously described.<sup>7</sup> The transducer operated at a frequency of 3.5 MHz with an average intensity of 0.045 mW/sq cm. Individual fetal breathing movements were identified on a video monitor and coded on a chart recorder by means of an event marker. The time during which gross fetal body movements occurred was indicated on the chart recorder with the use of a second event marker. Analysis of fetal breathing movements and gross fetal body movements during successive 5-minute intervals was performed on line by a PDP 11 computer and a program which measured fetal breathing movements and

gross fetal body movements to within 100  $\mu$ sec in real time.<sup>13</sup>

Measurement of the time during which fetal breathing movements occurred did not include episodes of apnea or gross fetal body movements. Apnea was defined as the absence of fetal breathing movements for 6 seconds or more and did not include episodes of gross fetal body movements.<sup>13</sup> Gross fetal body movements were identified as fetal activity sufficient to prevent recognition of fetal breathing movements. Portions of records which could not be analyzed because of technical failure or patient interruptions were not included in this analysis and made up 9.1% of total time for fetal heartbeats and 4.3% of total time for the analysis of fetal breathing movements and gross fetal body movements.

Results were presented as grouped means and standard errors of the mean. Significance was determined by the use of a *t* test for small samples with variances not assumed to be equal as described by Bailey.<sup>14</sup> The standard deviation of heartbeat intervals was presented as the median value and the range of values about the median.

### Results

The mean fetal heart rate during the 3-hour study period in 14 fetuses was  $138 \pm 0.4$  bpm. The mean fetal heart rates 5, 15, 30, and 90 minutes before exter-

\*ADR Ultrasound, Tempe, Arizona.



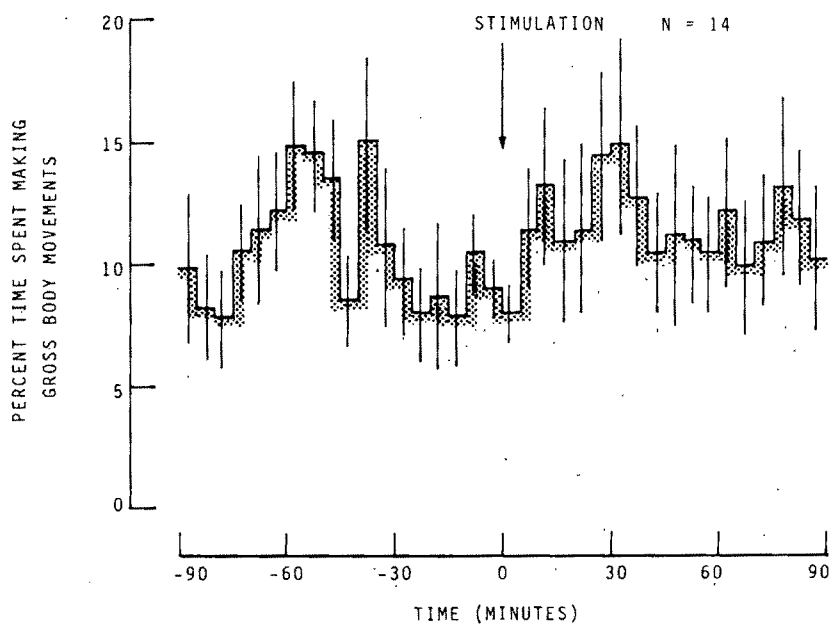


Fig. 4. The percentage of time spent making gross fetal body movements ( $\pm$ SEM) by 14 fetuses plotted in successive 5-minute intervals 90 minutes before and following external physical stimulation.

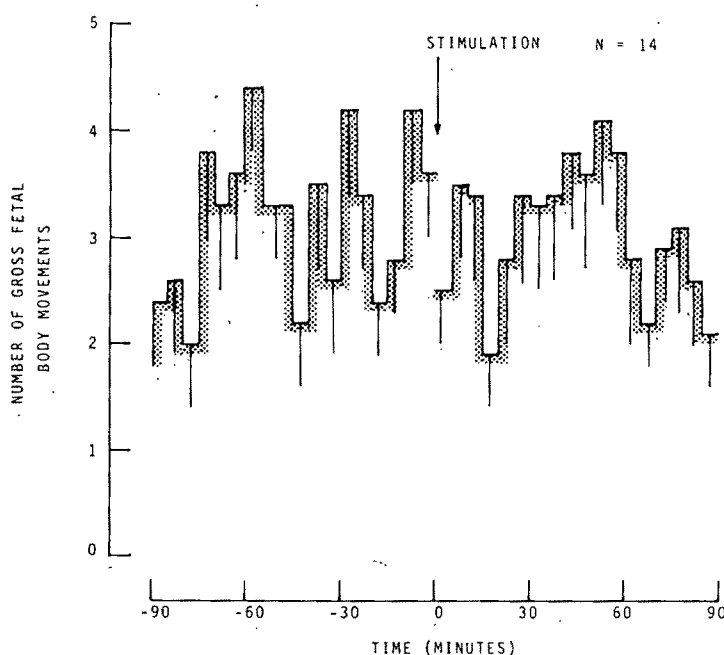


Fig. 5. The mean number of gross fetal body movements ( $\pm$ SEM) by 14 fetuses was plotted in successive 5-minute intervals before and following stimulation.

nal physical stimulation were compared to respective intervals of 5, 15, 30 and 90 minutes following external physical stimulation and no significant differences were observed (Fig. 2). The median value for the 5-minute SD of successive fetal heartbeat intervals was 30.3 msec (range 3.8 to 64.2 msec) over the 3-hour study and an

analysis comparing intervals of 5, 15, 30, and 90 minutes before external physical stimulation to respective intervals of 5, 15, 30, and 90 minutes following external physical stimulation did not show any significant differences (Fig. 3). The three fetuses who were stimulated every 15 minutes also showed no significant

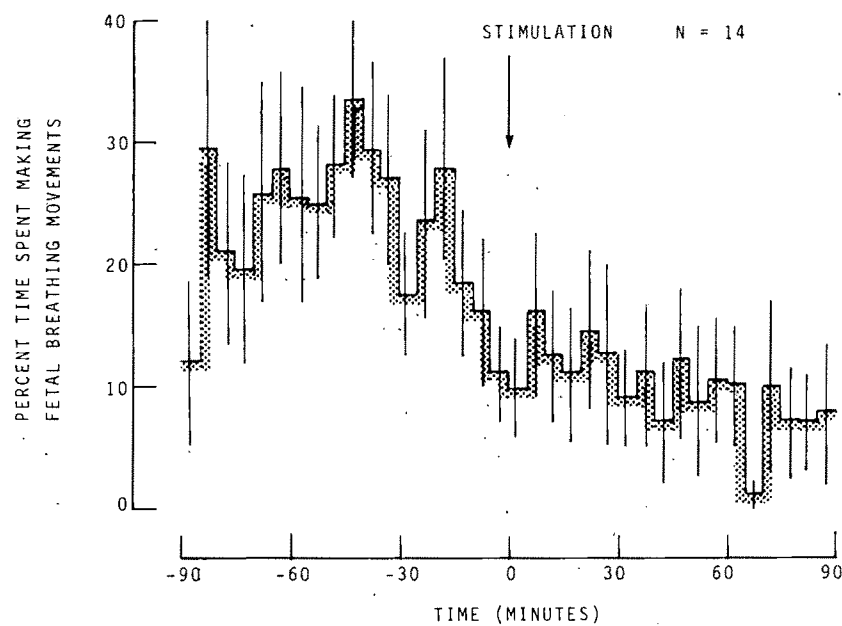


Fig. 6. The mean percentage of time spent making fetal breathing movements (±SEM) by 14 fetuses was plotted in successive 5-minute intervals 90 minutes before and following external physical stimulation.

Table I. Relationship of fetal heart rate accelerations and decelerations to gross fetal body movements

|                                        | Mean No. of gross fetal body movements with fetal heart rate |                      |                       | Mean No. of fetal heart rate accelerations with no gross fetal body movements (% of all accelerations) |
|----------------------------------------|--------------------------------------------------------------|----------------------|-----------------------|--------------------------------------------------------------------------------------------------------|
|                                        | Accelerations (%)                                            | Decelerations (%)    | No change (%)         |                                                                                                        |
| 90-minute control period               | 22.4 ± 3.8<br>(58.3%)                                        | 0.6 ± 0.5<br>(1.4%)  | 14.4 ± 1.8<br>(40.3%) | 8.6 ± 2.7<br>(23.3%)                                                                                   |
| 90-minute period following stimulation | 20.6 ± 3.6<br>(58.9%)                                        | 0.1 ± 0.09<br>(0.4%) | 14.3 ± 2.8<br>(40.7%) | 4.7 ± 1.1<br>(18.1%)                                                                                   |
| Significance                           | NS                                                           | NS                   | NS                    | NS                                                                                                     |

NS: Not significant.

change in heart rate or 5-minute SD when 5-minute intervals following stimulation were compared to 5-minute intervals preceding stimulation. Therefore, external physical stimulation did not produce a significant change in fetal heart rate or the 5-minute SD of fetal heartbeat intervals.

Gross fetal body movements consisted of fetal rolling and stretching movements and these occurred  $11.1\% \pm 0.4\%$  of the time on average. The percentage of time spent making gross fetal body movements by fetuses 5, 15, 30, and 90 minutes before external physical stimulation was not significantly different when compared to the time during intervals of 5, 15, 30, and 90 minutes following stimulation (Fig. 4). Fetuses made  $3.7 \pm 0.5$  and  $10.6 \pm 1.4$  gross fetal body movements during the 5- and 15-minute intervals immediately before stimulation, which were not significantly different from the  $2.2 \pm 0.6$  and  $8.8 \pm 1.6$  movements which occurred 5

and 15 minutes, respectively, after external physical stimulation (Fig. 5). The three patients who were stimulated every 15 minutes during the 3-hour study period also showed no significant change in the mean percentage of time spent making gross fetal body movements when 5-minute intervals before were compared to 5-minute intervals following fetal stimulation. Therefore, external physical stimulation of fetuses did not result in changes in either the percentage of time spent making gross fetal body movements or the number of movements made by fetuses.

Fetal heart rate accelerations were defined as episodes of increases in fetal heart rate from the baseline of 15 bpm or more lasting at least 15 seconds and decelerations in fetal heart rate were defined as decreases in fetal heart rate from the baseline of 15 bpm or more lasting at least 15 seconds. For the purposes of this part of the analysis gross fetal body movements which were



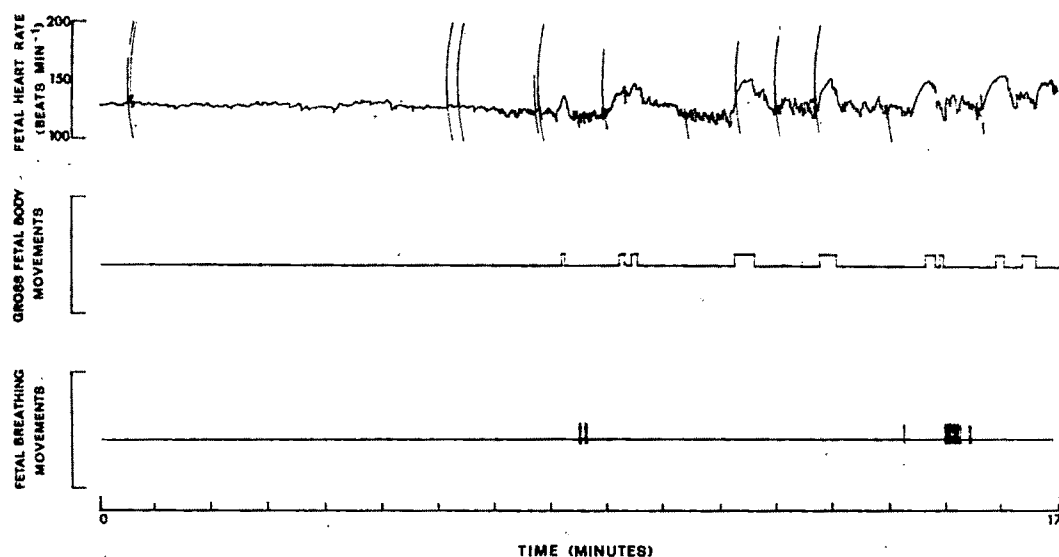


Fig. 7. A chart recording of fetal heart rate, gross fetal body movements, and fetal breathing movements over a 17-minute observation period. From 0 to 8 minutes there were no gross fetal body movements and the fetal heart rate demonstrated no significant accelerations or decelerations. From 8 to 17 minutes a normal episode of gross body movements accompanied by accelerations in fetal heart rate occurred.

separated by less than 12 seconds were considered as a single movement. During the 90-minute control period in 14 fetuses, 58.3% of gross fetal body movements were associated with acceleration in fetal heart rate, 1.4% of gross fetal body movements were associated with deceleration in fetal heart rate, and 40.3% of gross fetal body movements were not associated with significant change in fetal heart rate. When all accelerations in fetal heart rate were analyzed, 23.3% were not associated with gross fetal body movements recognized by our criteria (Table I). The relationship between gross fetal body movements and fetal heart rate change was not different during the 90-minute interval following stimulation, and the percentage of fetal heart rate accelerations not associated with gross fetal body movement was not significantly different. Therefore, external physical stimulation did not change the number of fetal heart rate accelerations and did not change the relationship of fetal heart rate accelerations to gross fetal body movements.

Individual fetal breathing movements occurred in episodes and were separated by periods of apnea during the control period and after external physical stimulation. The mean rate of fetal breathing movements during episodes of breathing was 48 breaths per minute and was not significantly different before or after stimulation. Fetuses made breathing movements  $16.6\% \pm 1.4\%$  of the time during the 3-hour studies. The mean percentage of time spent breathing by

fetuses diminished significantly over the 180-minute observation period, from  $23.4\% \pm 4.5\%$  during the control period to  $10.1\% \pm 3.8\%$  during the experimental period ( $P < 0.05$ ) (Fig. 6). However, the decrease in fetal breathing movements began prior to stimulation and there was no significant change in the percentage of time spent breathing when 5- and 15-minute intervals were compared immediately before and after stimulation. The three fetuses who were stimulated every 15 minutes also showed no significant change in fetal breathing activity when 5-minute intervals following stimulation were compared to 5-minute intervals preceding stimulation.

Examination of chart recordings of fetal heart rate, gross fetal body movements, and fetal breathing movements in individual patients demonstrated episodes of increased gross fetal body movements with accelerations in fetal heart rate, and these episodes were often separated by episodes of decreased or absent gross fetal body movements, during which time fetal heart rate recordings contained almost no accelerations or decelerations (Fig. 7). Episodes of increased gross fetal body movements and episodes of decreased gross fetal body movements did not seem to be predictably altered by external physical stimulation. Fig. 8 represents one study of external physical stimulation which was carried out during a time of decreased gross fetal body movements. It can be seen that stimulation did not appear to change the fetal activity pattern or

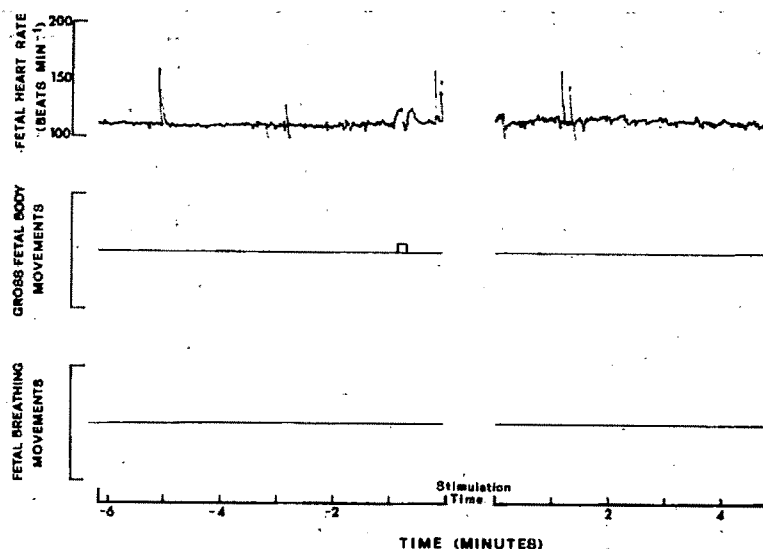


Fig. 8. A continuous chart recording interrupted by external physical stimulation demonstrated that stimulation did not seem to change patterns in heart rate, gross body movements, or fetal breathing movements during this period of fetal inactivity.

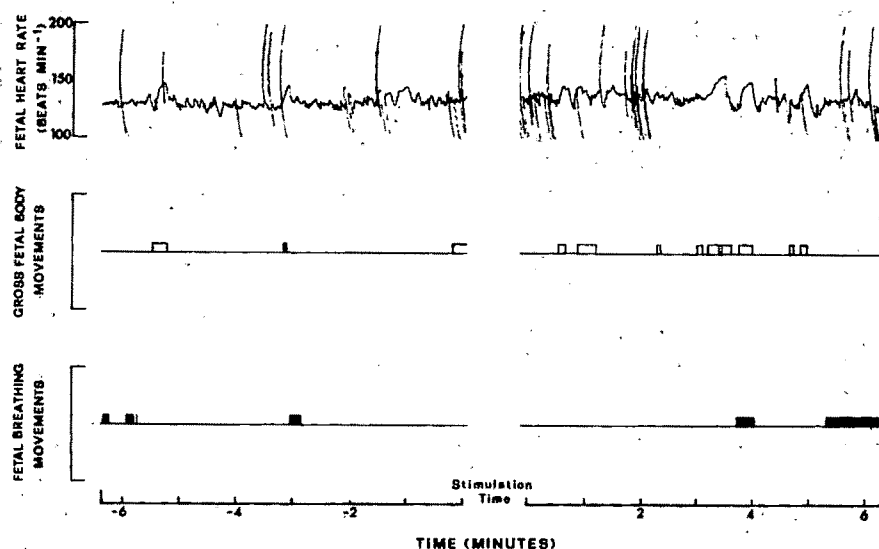


Fig. 9. A continuous recording of fetal heart rate, gross body movements, and breathing movements interrupted by external physical stimulation demonstrated no apparent change in fetal heart rate or movement patterns in response to stimulation during this period of fetal activity.

the fetal heart rate pattern. Fig. 9 represents another study when external physical stimulation was carried out during a time when the fetus was making breathing movements and gross fetal body movements and stimulation did not appear to alter the pattern of fetal activity. Occasionally, patterns of gross fetal body movements would change at the time of stimulation. Fig. 10 demonstrates a study which was performed during a period of increased gross fetal body movements and shows that following external physical stimulation gross

fetal body movements and accelerations in the fetal heart rate recording were diminished. The chart recordings in Figs. 7, 8, 9, and 10 suggest that patterns of fetal activity and inactivity are part of the normal physiology of fetal life and that vigorous external physical stimulation does not predictably alter these patterns.

#### Comment

This study did not confirm reports that fetal activity patterns are increased following external physical



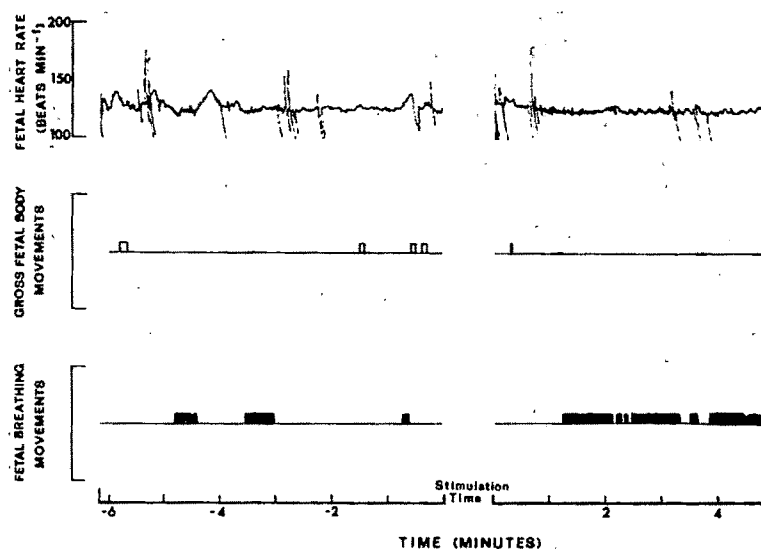


**Fig. 10.** A continuous recording of fetal heart rate, gross body movement, and breathing movements interrupted by external physical stimulation demonstrated that there was an apparent decrease in gross fetal body movements and accelerations in heart rate accompanying gross fetal body movements following external physical stimulation. There was no change in fetal breathing activity following stimulation.

stimulation.<sup>2, 3</sup> We found no consistent changes in fetal heart rate, fetal heart rate variability, gross fetal body movements, and accelerations in fetal heart rate with movement following vigorous external physical stimulation of healthy fetuses. The data suggested that changes from inactive antepartum fetal heart rate patterns to active patterns after stimulation were most probably due to an increase in the length of observation time rather than to the stimulation itself. We previously measured fetal breathing movements and gross fetal body movements continuously over 24-hour observation periods and reported that gross fetal body movements occurred in episodes lasting 20 to 60 minutes which occurred every 60 to 90 minutes.<sup>7</sup> Campbell and associates<sup>15</sup> demonstrated, using mathematical time domain and spectral methods, that gross fetal body movements were not random. Furthermore, Brown and associates<sup>16</sup> recently analyzed 1,069 nonstress tests in 333 high-risk hospitalized pregnant women. These investigators conducted nonstress tests for up to 120 minutes and found that, in fetuses with good outcomes, there was a 23% incidence of nonreactive tests after 20 minutes following the onset of each study but only 4% of tests were nonreactive after 60 minutes. Brown and associates found that all fetuses who were healthy at birth were reactive by 80 minutes and that all fetuses who were nonreactive for 120 minutes subsequently demonstrated severe asphyxia in labor and at birth or died in utero.

The present study demonstrated that neither the

mean fetal heart rate nor the fetal heart rate variability, quantified as the 5-minute SD of successive heartbeat intervals, was significantly altered by external physical stimulation. Aladjem and associates<sup>17</sup> noted that external pressure applied to the fetal head or neck elicited an increase in fetal heart rate over baseline of approximately 13% lasting 27 seconds. Our study did not show a significant increase in the mean fetal heart rate during the 5 minutes following stimulation but it is possible the acute fetal heart rate response to external physical stimulation may be too short-lived to reach significance when 5-minute time intervals are measured.

The mean percentage of time spent making fetal breathing movements was  $16.6 \pm 1.4$  over the 3-hour observation period and decreased from  $23.4 \pm 4.5$  during the 90-minute control period to  $10.1 \pm 3.8$  during the 90-minute period following stimulation. The decrease in fetal breathing movements occurred during the control period prior to stimulation and there was no significant difference between fetal breathing movements measured 5 and 15 minutes before stimulation compared to 5 and 15 minutes following stimulation. Natale and associates<sup>18</sup> have reported a decrease in fetal breathing movements in pregnant women who fasted at 32 to 34 weeks' gestational age from 20.6% at 0800 to 1000 hours to 17.2% at 1000 to 1300 hours.<sup>18</sup> The decrease in fetal breathing activity in the present study began prior to stimulation and may reflect a normal pattern in fasting women at term gestation.

This study demonstrated that normal behavior pat-

terns of healthy fetuses are not predictably altered by external physical stimulation. This suggests that either fetal behavior patterns are not altered by vigorous external physical stimulation or external physical manipulation is not an adequate stimulus to change fetal rest-activity patterns. It will be important to account for rest-activity patterns when fetal health is monitored and to recognize, even in healthy fetuses, that external

physical stimulation does not reliably produce fetal activity when measured with real-time ultrasonic scanners.

We wish to thank Drs. Bob Brown, John Challis, Paul Harding, and Renato Natale for their interest in this work and Mrs. T. Clarke and T. Vandenberg for their excellent technical assistance.

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# Demonstration of novel compounds in human fetal tissues and a consideration of their possible role in parturition

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A study of a new group of compounds and their possible relevance to human parturition is presented. These compounds behave chemically and chromatographically like steroids and have been tentatively identified as analogues of 11-hydroxypregnenolone. Determined by radioenzymatic assay, highest levels were found in the fetal zone of the fetal adrenal and were about 100 times those of fetal serum. Umbilical cord levels were higher after spontaneous labor than after elective cesarean section or induced labor in premature as well as mature infants. It is hypothesized that a fetal factor distinct from adrenocorticotrophic hormone acts on the adrenal cortex, particularly the fetal zone, to stimulate 11 $\beta$ -hydroxylation of nonglucocorticoid corticosteroids and that these steroids and their metabolites are excreted via the urine into the amniotic fluid. Thus they would reach the membranes and decidua where they might compete with progesterone for receptor sites, decreasing lysosomal stability with the resultant production of prostaglandins and the onset of labor. (AM. J. OBSTET. GYNECOL. 139:353, 1981.)

ALTHOUGH PREMATURE LABOR is a major cause of potentially preventable neonatal mortality, virtually nothing is known about its etiology. Considerable progress has been made in understanding the events leading to the initiation of parturition in the sheep and other animals, but in primates the mechanism appears to be different and its investigation is hampered by ethical and other problems. The fetal adrenal production of cortisol is considered to play a major role in the initiation of labor in ruminants and to be important in fetal maturation in all species.<sup>1, 2</sup>

In studying the metabolism of cortisol in the human fetus, it was found that a steroid-like inhibitor(s) of the conversion of cortisol to cortisone, its chief metabolite, was present in high concentrations in fetal serum at delivery.<sup>3</sup> This paper represents a study of this material

and its possible significance with respect to human parturition.

## Subjects and methods

Subjects included infants delivered at the Montreal General Hospital and referrals from local hospitals to the Montreal Children's Hospital. Fetal tissues were obtained at hysterotomy for maternal indications and at autopsy of newborn infants dying of hyaline membrane disease or congenital heart disease. Placental tissue and umbilical cord serum were obtained at delivery. The cord blood used was that routinely collected at delivery (mixed arterial and venous blood). If not required for clinical purposes, it was centrifuged and the serum was frozen for assay.

The inhibitory material was measured by a radioenzymatic assay (REA) developed for this purpose, with the use of the 11-hydroxysteroid dehydrogenase (11-HSD) of the human placenta (the enzyme which converts cortisol to cortisone) and tritiated cortisol. The amount of substrate (or inhibitor) measured using nonradioactive cortisol standards and, therefore, expressed as cortisol equivalents is proportional to its inhibition of the conversion of tracer cortisol to cortisone under standardized conditions.<sup>4</sup> A typical standard curve is shown in Fig. 1. Cortisol and corticosterone

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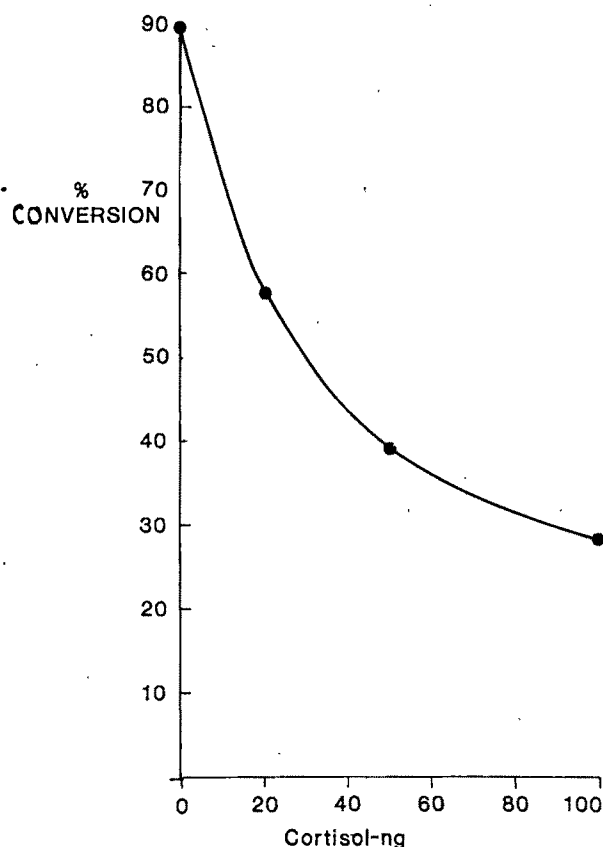


Fig. 1. Standard curve for REA. Conversion of  $^3\text{H}$ -cortisol to cortisone is plotted against the amount of cortisol added. The unknown is read from the curve according to the conversion obtained in its presence. The greater the amount of unknown which competes for conversion, the lesser the amount of tracer converted. Since the identity of the competitors is not established, the result is expressed in cortisol equivalents. Each point is the mean of duplicate values.

were measured as described previously.<sup>5, 6</sup> Antibodies were obtained from commercial sources and used according to the instructions.

### Results

**Nature of the inhibitory material.** Previous studies showed that the inhibitor(s) was dialyzable, ethyl acetate extractable, and heat stable.<sup>3</sup> Since these characteristics were suggestive of a steroid-like substance, studies were carried out to determine whether the inhibitor might be competitive in type. Lineweaver-Burke plots of extracts of serum, adrenal, and liver were characteristic of a competitive type of inhibition (Fig. 2), so that it seemed likely that one or more  $11\beta$ -hydroxysteroids ( $11\text{-HCS}$ ) were responsible.

**Source of the inhibitory material.** The inhibitory material was found in highest concentrations in the neonatal adrenal (Table I), where it was about 100

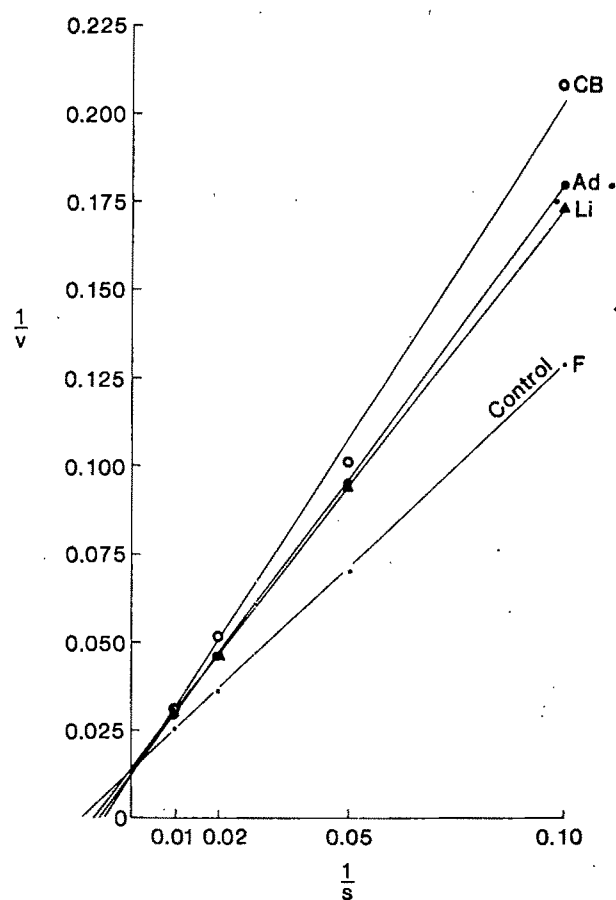


Fig. 2. Lineweaver-Burke plots to show the nature of the inhibition of cortisol to cortisone conversion in placental homogenate.  $v$  = Velocity;  $s$  = substrate concentration. The inhibitors were extracts of pooled umbilical mixed cord serum (CB, open circles), liver (Li, solid triangles) from a premature infant dying of hyaline membrane disease and adrenal tissue (Ad, solid circles) from a term infant dying of congenital heart disease. Each point is the mean of duplicate samples.

times the fetal serum concentration. Concentrations were also high in the liver and were increased over serum concentrations in the lung, heart, and kidney. Little activity was found in the spleen, brain, thymus, or intestine.

**Differentiation from known fetal  $11\text{-hydroxysteroids}$ .** All the material in maternal serum was accounted for as cortisol and corticosterone (Table I), whereas the bulk of the material in the fetus could not be accounted for as glucocorticoids.

**Chromatographic separation on Sephadex LH-20.** Ethyl acetate extracts chromatographed on columns of Sephadex LH-20 39 cm long showed a major peak of inhibitory activity, as measured by REA, intermediate in polarity between corticosterone and cortisol (Fig. 3). This was the only significant peak seen in the fetal zone



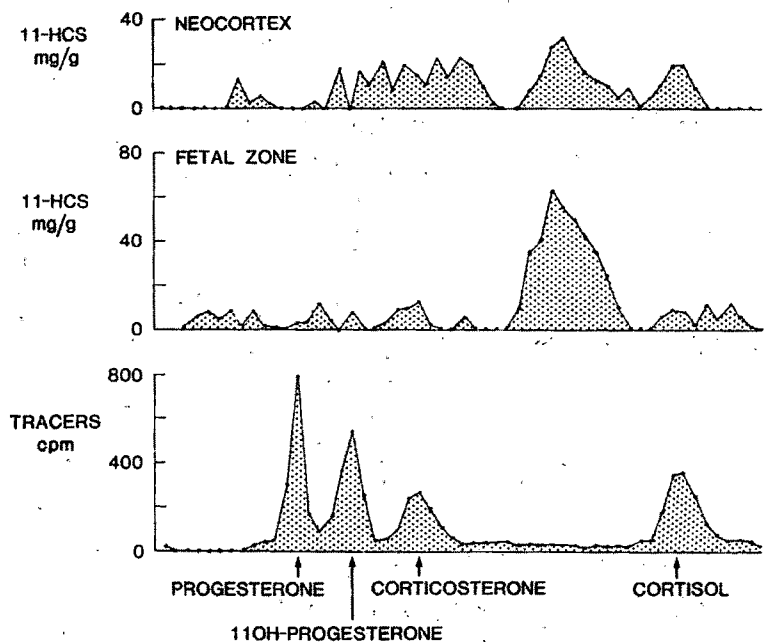


Fig. 3. Chromatograms of the fetal zone and neocortex of the adrenal of an infant dying of congenital heart disease shortly after a normal birth. Radioactive tracers were added to indicate the positions of cortisol, corticosterone, progesterone, and 11 $\beta$ -hydroxyprogesterone. 11-HCS were assayed by REA. Aliquots of the fractions were also assayed by means of the various systems in Table II.

Table I. 11-HCS levels in various tissues (mean  $\pm$  SE, ng/ml or ng/gm)

| Subject | Tissue     | Gestational age (weeks) | Labor  | n  | Total 11-HCS | Cortisol     | Corticosterone | NGCS         |
|---------|------------|-------------------------|--------|----|--------------|--------------|----------------|--------------|
| Mother  | Serum      | 40.9 $\pm$ 0.9          | Spont. | 6  | 450 $\pm$ 40 | 445 $\pm$ 45 | 10 $\pm$ 5     | 0            |
| Fetus   | Serum      | 40.0 $\pm$ 1.2          | Spont. | 15 | 269 $\pm$ 17 | 91 $\pm$ 10  | <10            | 169 $\pm$ 18 |
| Fetus   | Serum      | 39.0 $\pm$ 0.2          | None   | 10 | 145 $\pm$ 38 | 49 $\pm$ 6   | <10            | 101 $\pm$ 36 |
| Fetus   | Serum      | 33.0 $\pm$ 1.2          | Spont. | 10 | 282 $\pm$ 38 | 42 $\pm$ 12  | <10            | 241 $\pm$ 47 |
| Fetus   | Serum      | 16-20                   | None   | 3  | 110 $\pm$ 13 | 7 $\pm$ 3    | <10            | 103 $\pm$ 15 |
| Fetus   | Adrenal    | 18                      | None   | 1  | 1,500        | 188          | <200           | 1,300        |
| Fetus   | Adrenal    | 29                      | None   | 1  | 3,900        | 315          | <200           | 3,600        |
| Fetus   | Fetal zone |                         |        |    | 27,000       | 1,100        | 700            | 24,150       |
| Fetus   | Neocortex  | 40                      | Spont. | 1  | 33,000       | 4,100        | 4,000          | 18,900       |

Spont. = Spontaneous onset; NGCS are expressed as cortisol equivalents, measured by REA; n = number of infants studied.

of the fetal adrenal, while a number of other small peaks were seen in the neocortex, two of which corresponded to cortisol and corticosterone.

**Radioimmunoassay studies of the major peak of inhibitory activity.** Antibodies were obtained which cross reacted with 11 $\beta$ -hydroxydehydroepiandrosterone and 11 $\beta$ -hydroxypregnenolone, and the results are shown in Table II. These studies suggested that this material might be 11 $\beta$ -hydroxypregnenolone; however, a third antibody did not support this conclusion and the possibility that the compound may be 11 $\beta$ -hydroxy, 20-dihydropregnenolone is being pursued. Isolation of larger amounts of material to be subjected to gas-liquid chromatography-mass spectrometry examination is in progress but so far has proved difficult

because of the lability of the compound after purification.

**Specificity of 11-HSD.** One would expect that if the inhibitor has a physiologic role, then it might be very potent in the reaction catalyzed by 11-HSD and that a survey of the specificity of the enzyme would be useful. Such studies are shown in Table III where it may be seen that the most potent competitors (or inhibitors) of the reaction are 11-hydroxylated progesterone and its analogues and 11-hydroxypregnenolone. This also provides suggestive evidence that the structure of the inhibitor is similar to that of 11-hydroxypregnenolone or a progesterone analogue. The formation of an  $\alpha$ -hydroxyl at C<sub>20</sub>, a prominent pathway of metabolism, has little effect whereas addition of hydroxyl groups at

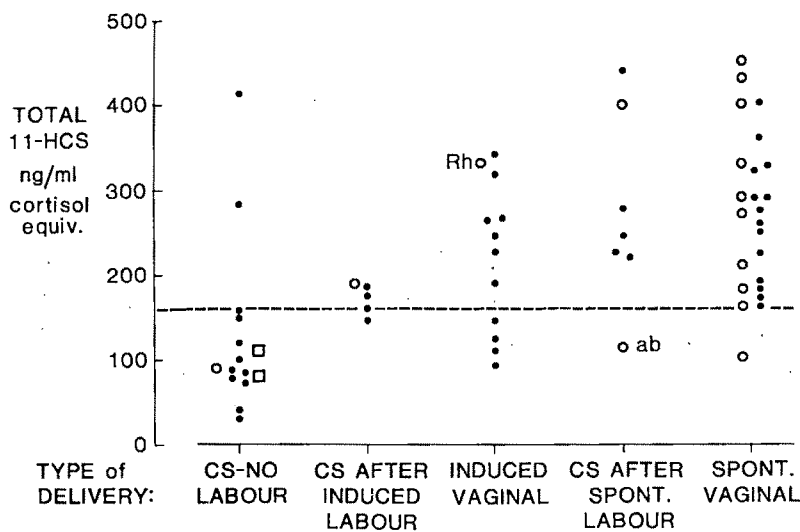


Fig. 4. Total 11-HCS in umbilical cord serum at delivery. Solid circles indicate infants at term (more than 37 weeks' gestation); open circles, premature infants; squares, midterm infants delivered by hysterotomy; CS, cesarean section; *spont.*, spontaneous labor; *ab*, a case of abruptio placentae with acute chorioamnionitis.

Table II. Comparative assays of the main peak isolated chromatographically from the fetal zone of a neonatal adrenal.

| Type  | Assay system   |                          | 11-Hydroxypregnenolone |       |
|-------|----------------|--------------------------|------------------------|-------|
|       | Tracer         | Binding protein          | Cr-Reax                | µg/gm |
| RTA   | Cortisol       | Horse transcortin        | 0.01                   | ND    |
| RTA   | Corticosterone | Monkey transcortin       | 0.01                   | ND    |
| RIA   | DHEA           | DHEA antiserum           | 0.01                   | ND    |
| RIA 1 | Progesterone   | Progesterone antiserum 1 | 2.0                    | 7.8   |
| RIA 2 | Progesterone   | Progesterone antiserum 2 | 5.0                    | ND    |
| REA   | Cortisol       | Placental HSD            | 400                    | 7.3   |

RTA = Radiotransin assay; RIA = radioimmunoassay; HSD = hydroxysteroid dehydrogenase; ND = not detected; Cr-Reax = cross-reactivity relative to that of the tracer steroid at 50% displacement; DHEA = dehydroepiandrosterone. Lack of detection of 11-hydroxypregnenolone by progesterone RIA 2 indicates that the unknown was a different compound.

C<sub>21</sub> (corticosterone) and C<sub>17</sub> (cortisol) progressively decreases activity. A 20β-hydroxy group decreases it considerably and the loss of the side chain (11-hydroxyandrostenedione and 11-hydroxydehydroepiandrosterone) does so still more. Activity is moderately reduced by saturation of the A-B rings and is greatly reduced by a 16α-hydroxy group, a C<sub>21</sub> sulfate, or a C<sub>18</sub> aldehyde.

**Significance of this material with respect to parturition.** Levels of total 11-HCS in individual cases are shown in Fig. 4. In cases where the onset of labor was spontaneous, only two of 31 infants had levels below 160 ng/ml; one of these occurred with abruptio placentae and acute chorioamnionitis. Of the 13 cases delivered without labor, only two had values above 160 ng/ml, while the group of 16 where labor was induced with oxytocin had intermediate values. Levels in two midterm fetuses were similar to those of the majority

without labor. The highest values of all (450 ng/ml) occurred in a 29-week-old infant born after precipitate labor of spontaneous onset (cause unknown) who died of the respiratory distress syndrome (RDS). Mean values for total 11-HCS were higher if the onset of labor was spontaneous than if labor was induced ( $P < 0.025$ ) or absent ( $P < 0.025$ ). The results in premature infants were similar to those in term infants.

When cortisol was considered separately (Fig. 5), it made up a larger fraction in the three groups at term (0.34 in each) than in the premature group (0.14). Levels of corticosterone, the other glucocorticoid present in cord serum, were consistently about 10% of those of cortisol and, therefore, very small compared to those of the remaining nonglucocorticoid fraction (NGCS). As shown in Fig. 5, NGCS were highest in the premature infants born after the spontaneous onset of labor, exceeding those of the term-spontaneous la-



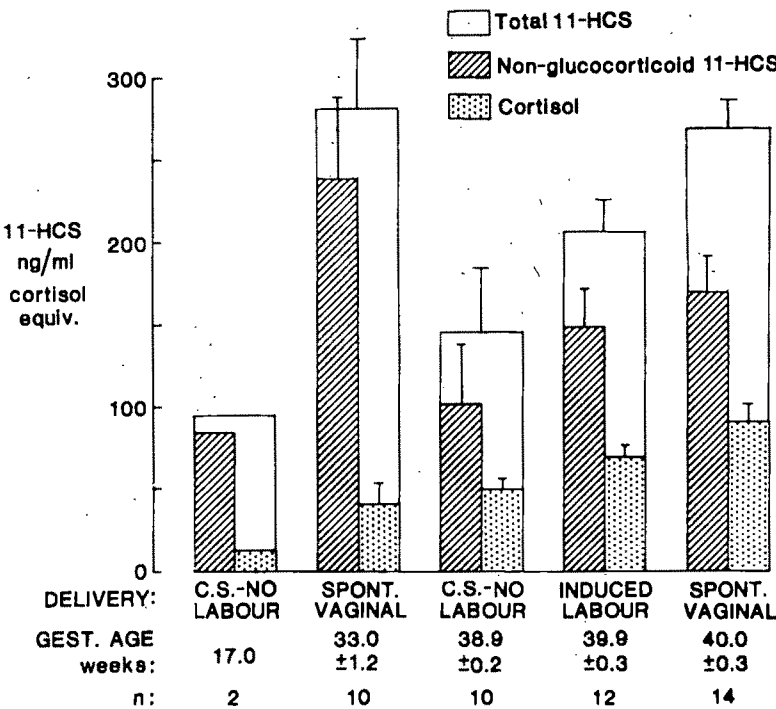


Fig. 5. Total 11-HCS, cortisol, and nonglucocorticoid 11-HCS in umbilical cord serum at delivery, means ± SE. Gestational age is indicated under each group.

bor group ( $P = 0.05$ ), the term-induced labor group ( $P < 0.01$ ) and the group with no labor ( $P < 0.001$ ). Considered individually, the values of the total 11-HCS appeared to be more closely related to the onset of labor than was either cortisol or NGCS alone.

Comment

These data suggest that an 11-HSD inhibitor, possibly an  $11\beta$ -hydroxypregnenolone analogue, produced by the fetal zone of the fetal adrenal, rises in close association with the onset of either premature or term labor. The observation that the values in the group without labor fall into two groups, most being very low, but a few being high, suggests that the rise occurs before labor commences. Thus, it may be the  $11\beta$ -hydroxylated corticosteroids of the fetal adrenal as a whole, rather than cortisol, which are involved in the onset of labor in the human.

Little attention has been paid to the nonglucocorticoid members of this group although  $11\beta$ -hydroxyandrostenedione and  $11\beta$ -hydroxyprogesterone have been noted in the adrenal venous blood of pigs and dogs in small amounts<sup>7</sup> and  $11\beta$ -hydroxydehydroepiandrosterone has been found in a human virilizing adrenal adenoma<sup>8</sup> and in the urine of infants in the first week of postnatal life.<sup>9</sup> In rats the adrenal response to moderate stress was inhibited by  $11\beta$ -hydroxyproges-

Table III. Relative 11-HSD inhibitory activity\*

|                                                                 |      |
|-----------------------------------------------------------------|------|
| 11 $\beta$ -Hydroxyprogesterone                                 | 600  |
| 11 $\beta$ -Hydroxypregnenolone                                 | 400  |
| 11 $\beta$ -Hydroxy-20 $\alpha$ -dihydroprogesterone            | 300  |
| Corticosterone                                                  | 250  |
| Cortisol                                                        | 100  |
| 11 $\beta$ -Hydroxy-20 $\beta$ -dihydroprogesterone             | 38   |
| 11 $\beta$ -Hydroxyandrostenedione                              | 14   |
| 11 $\beta$ -Hydroxydehydroepiandrosterone                       | 12   |
| 11 $\beta$ -Hydroxy-5 $\alpha$ -androstane-3 $\beta$ -ol-17-one | 9    |
| 11 $\beta$ -Hydroxyandrosterone                                 | 4    |
| 11 $\beta$ -Hydroxy-5 $\beta$ -androstane-3 $\beta$ -ol-17-one  | 4    |
| 11,16 $\beta$ -Hydroxydehydroepiandrosterone                    | 4    |
| 6 $\beta$ -Hydroxycortisol                                      | <0.1 |
| Cortisol sulfate                                                | 5    |
| Aldosterone                                                     | <0.1 |

\*Calculated at 50% displacement of tracer cortisol in placental REA.

terone.<sup>10</sup> However, these steroids were present in much smaller amounts than the principle steroid assayed, thought possibly to be  $11\beta$ -hydroxy, 20-dihydropregnenolone. Both  $11\beta$ -hydroxyprogesterone and  $11\beta$ -hydroxypregnenolone are strong competitors for 11-HSD, as are their 20 $\alpha$ -dihydro derivatives. 11-HSD has been regarded as relatively nonspecific; however, it has not previously been recognized that it has a much higher affinity for these unusual steroids than for cortisol. Thus, this enzyme may be directed primarily at these substrates rather than at cortisol, although there

seems little doubt that the enzyme also performs an important function in protecting the developing fetus from excessive amounts of glucocorticoids which might be expected to retard growth.

Might the rise in 11-HCS not be merely a consequence of labor rather than a causative factor? While it is difficult to rule this out, the low cortisol levels in premature infants suggest that intrapartum stress is not a factor since such infants are able to respond to stress immediately after birth with normal elevations of cortisol.<sup>11</sup>

For many years the large mass of the human fetal adrenal, composed mainly of the unique fetal zone, remained an enigma. The finding that a hitherto unrecognized steroid is produced by this zone in large amounts in association with labor suggests that the fetal zone may play a role in parturition. How might this steroid act to bring about labor? It seems possible that this tissue, under the influence of some factor other than adrenocorticotrophic hormone, may have as its

primary function the production of an 11 $\beta$ -hydroxysteroid which with its metabolites passes directly to the placenta and via the urine and amniotic fluid to the membranes and decidua. Here there is evidence to suggest that it would be converted to a progesterone analogue since the conversion of the double bond at C<sub>5</sub> to C<sub>4</sub> is known to occur in the placenta and endometrium<sup>13, 14</sup>; such a substance might be expected to compete for progesterone receptors.<sup>15</sup> Such competition might result in a decreased effective progesterone concentration in the decidua which in turn would result in increased sensitivity to oxytocin, increased prostaglandin production, and the onset of labor. Although much work remains to substantiate these events, this would seem to provide a reasonable working hypothesis.

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# Adrenal responses to prostaglandin E<sub>2</sub> in newborn lambs

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Prostaglandin E<sub>2</sub> (PGE<sub>2</sub>) is present in fetal sheep and stimulates adrenal steroidogenesis in the fetal and newborn sheep. We have examined the site of this prostaglandin effect and the changes in adrenal responsiveness to exogenous PGE<sub>2</sub> and adrenocorticotrophic hormone (ACTH) in in vivo studies with newborn lambs. A bolus injection of PGE<sub>2</sub> (50 µg) into the brachiocephalic trunk or descending aorta raised plasma cortisol (F)  $25.3 \pm 4.5$  (SE) ng/ml to  $75.5 \pm 5.4$  ng/ml at 15 to 30 minutes and  $23.2 \pm 3.0$  to  $60.5 \pm 7.1$  ng/ml at 15 to 30 minutes, respectively (both  $P < 0.001$ ). Intra-aortic infusion of PGE<sub>2</sub> (1.25 µg/min) produced a similar significant rise in F. Basal plasma F decreased from  $34.3 \pm 7.4$  ng/ml at 9 to 11 days to  $14.1 \pm 4.0$  ng/ml at 12 to 13 days and  $18.2 \pm 5.7$  ng/ml at 17 to 18 days. Peak F during a 60-minute PGE<sub>2</sub> infusion was significantly greater at 9 to 11 days ( $127.3 \pm 14.6$  ng/ml) than at the two later times ( $57.2 \pm 7.07$  and  $55.7 \pm 14.6$  ng/ml, respectively, both  $P < 0.01$ ). The response to ACTH was greater 24 hours after birth than at 7 or 28 days of age. Injection of ACTH or  $\alpha$ -melanocyte-stimulating hormone during an intra-aortic PGE<sub>2</sub> infusion did not further elevate plasma F over the rise seen with PGE<sub>2</sub> alone. The results suggest both direct and indirect stimulatory effects of PGE<sub>2</sub> on adrenal function in newborn sheep, and are consistent with a role of endogenous PGE<sub>2</sub> as an intermediate factor in ACTH action. (AM. J. OBSTET. GYNECOL. 139:359, 1981.)

GLUCOCORTICOID secreted by the fetal adrenal gland has a well-established role in the initiation of parturition in the sheep<sup>1</sup> and in the preparation for extrauterine life in several species.<sup>2</sup> The fetal cortisol concentration rises in late gestation in the sheep<sup>3</sup>; however, the regulation of the fetal adrenal gland remains poorly understood. The response of the gland to adrenocorticotrophic hormone (ACTH) increases during late gestation<sup>4</sup> and fetal ACTH concentrations increase before birth,<sup>5</sup> but the possibility that other substances affect adrenal function at this time has not been excluded.

Prostaglandins are present in the circulation of the fetus and the newborn lamb.<sup>6</sup> Stimulation of cortisol

secretion by prostaglandin E<sub>2</sub> (PGE<sub>2</sub>) has been observed in the fetal sheep<sup>7</sup> and in the newborn lamb.<sup>8</sup> In this study in the newborn lamb we have examined the site of action of PGE<sub>2</sub> and the relationship of its effect to that of ACTH and  $\alpha$ -melanocyte-stimulating hormone ( $\alpha$ -MSH), another possible adrenal trophic factor, in the fetus and newborn lamb. We have compared the changes in responsiveness of the adrenal gland to PGE<sub>2</sub> and ACTH with advancing postnatal age. It is suggested that PGE<sub>2</sub> acts upon the brain and/or the pituitary as well as at the level of the adrenal gland, where its action may be as an intermediate in ACTH action.

## Material and methods

**Surgical preparation.** Newborn lambs of mixed breed which were born spontaneously at term were used in these experiments. When the lambs were 5 to 7 days old, vascular catheterization with a Fr 3½ umbilical artery catheter (Sherwood Industries, St. Louis, Missouri) was performed with the lambs under local anesthesia. One catheter was introduced through the median artery in the forelimb and positioned so that its tip was lying in the brachiocephalic trunk. (In the sheep, arterial supply to the forelimbs and carotid

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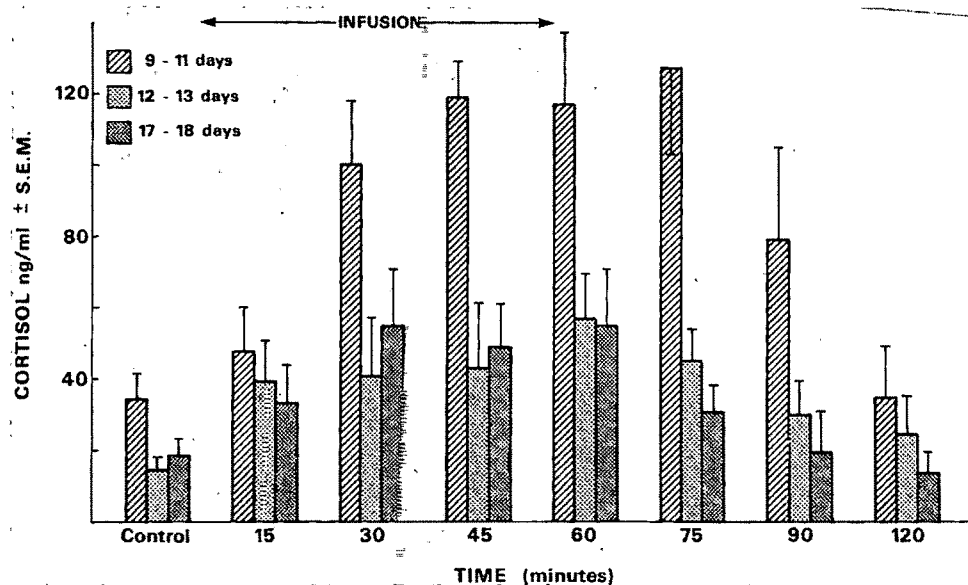


Fig. 1. Effect of postnatal age on basal and PGE<sub>2</sub>-stimulated cortisol concentrations.

supply to the head arise from a single brachiocephalic trunk.) A second catheter was introduced by the circumflex femoral artery and positioned so that its tip lay in the distal thoracic aorta. Initial catheter position was confirmed in each case by x-ray film and maintenance of catheter position was confirmed at the end of the experiment by x-ray film or dissection at autopsy. Catheters were filled with heparin and saline (100 U/ml). The lambs remained with their mother except during experimental periods. Each lamb appeared healthy and was gaining weight during the period of experimental observations.

**Experimental procedures.** Experiments were carried out between 0800 and 1300 hours, beginning 2 days after operation and every second day thereafter. The animals were resting quietly in cloth slings in an isolated laboratory. Thirty minutes was allowed for acclimatization to the sling, after which various experiments were carried out as described below.

**Prostaglandin bolus injections.** Ten lambs at age 8 to 12 days received a 50 µg bolus of PGE<sub>2</sub> (The Upjohn Co.) in 1 ml of saline injected into the distal thoracic aorta. Three animals at age 13 to 16 days received a similar bolus injection into the brachiocephalic trunk.

**Prostaglandin infusions.** PGE<sub>2</sub> was infused at a dose of 1.25 µg/min into the thoracic aorta, by means of an infusion pump (Harvard Instruments) at 0.247 ml/min, for 60 minutes. Infusions were performed in eight animals at 9 to 11 days, in seven animals at 12 or 13 days, and in five animals at 17 or 18 days. Five animals at 9 or 10 days also received an infusion of PGE<sub>2</sub> at 1.25 µg/min for 15 minutes.

Seven of the animals used in the prostaglandin bolus

and infusion experiments received a 1 ml bolus injection of saline into the thoracic aorta as a control in a procedure similar to that of the prostaglandin bolus injection. This was done at age 10 to 14 days and is presented with the prostaglandin administration data in Table I.

**ACTH bolus injections.** Five lambs received ACTH at 4 hours and 24 hours; six lambs received ACTH at 1 week; eight received ACTH at 1 to 2 months, and five received ACTH as adults. As a control for this portion of the study a 1 ml saline injection was given to four lambs at 24 hours, to five lambs at 1 week, and to four lambs at 1 to 2 months. This experiment and related control data are presented in Table II. None of these animals was used in the prostaglandin experiments.

**Simultaneous ACTH or α-MSH and PGE<sub>2</sub>.** Four lambs aged 8 to 9 days received a bolus injection of 10 µg of ACTH into the brachiocephalic trunk 30 minutes after the start of a saline infusion (0.247 ml/min) into the thoracic aorta for 60 minutes. The ACTH bolus injection was repeated in three of these lambs at age 12 or 13 days at the same time (i.e., 30 minutes after start of infusion) during a PGE<sub>2</sub> infusion at 1.25 µg/min for 60 minutes. Another four lambs were given a bolus injection of 100 µg of α-MSH during a saline infusion at age 8 or 9 days; three of these lambs received another injection of 100 µg of α-MSH during a PGE<sub>2</sub> infusion at age 12 or 13 days.

**Sampling protocol.** Blood samples of 1 ml were taken with the use of the brachiocephalic artery catheter (femoral artery in the ACTH bolus group) in each of the above protocols at times -30, -15, 0, +15, +30, +45, +60, +90, and +120 minutes relative to the bolus



**Table I.** Cortisol responses to various prostaglandin stimulations

| Experimental group                                      | Cortisol ng/ml ( $\pm$ SEM) |                  |                  |                  |                   |                 |                 |                |
|---------------------------------------------------------|-----------------------------|------------------|------------------|------------------|-------------------|-----------------|-----------------|----------------|
|                                                         | Mean basal                  | +15              | +30              | +45              | +60               | +75             | +90             | +120           |
| 50 $\mu$ g bolus of PGE <sub>2</sub> , brachio-cephalic | 25.1 $\pm$ 4.5              | 76.7 $\pm$ 7.5*  | 74.3 $\pm$ 9.2*  | 50.0 $\pm$ 7.2*  | 21.3 $\pm$ 4.8    |                 | 60 $\pm$ 0.5    | 35 $\pm$ 16.4  |
| 50 $\mu$ g bolus of PGE <sub>2</sub> , aortic           | 23.2 $\pm$ 3.0              | 59.3 $\pm$ 11.0* | 66.0 $\pm$ 9.7*  | 42.3 $\pm$ 8.4*  | 38.6 $\pm$ 7.9†   |                 | 32.1 $\pm$ 5.4  | 35.0 $\pm$ 4.8 |
| 1.25 $\mu$ g/min of PGE <sub>2</sub> for 15 min         | 27.2 $\pm$ 4.9              | 95.4 $\pm$ 5.2*  | 72.2 $\pm$ 7.0*  | 47.5 $\pm$ 6.2*  | 39.8 $\pm$ 8.6    |                 |                 |                |
| 1.25 $\mu$ g/min of PGE <sub>2</sub> for 60 min         | 26.9 $\pm$ 4.1              | 48.1 $\pm$ 9.4*  | 58.9 $\pm$ 14.1* | 57.5 $\pm$ 14.4* | 68.3 $\pm$ 10.4*† | 53.5 $\pm$ 8.2* | 31.9 $\pm$ 8.3  | 24.0 $\pm$ 8.1 |
| Saline control                                          | 34.3 $\pm$ 5.8              | 40.3 $\pm$ 11.9  | 36.3 $\pm$ 8.7   | 29.1 $\pm$ 4.6   | 37.1 $\pm$ 11.0   |                 | 35.9 $\pm$ 14.7 | 43.0 $\pm$ 6.5 |

\*p < 0.001, compared to basal.  
†p < 0.05, when the two 60-minute values are compared with each other.

**Table II.** Effect of postnatal age upon cortisol response to ACTH

| Group       | Plasma cortisol (ng/ml) ( $\pm$ SEM) |                  |                  |                   |                   |                  |                  |
|-------------|--------------------------------------|------------------|------------------|-------------------|-------------------|------------------|------------------|
|             | Mean basal                           | +15              | +30              | +45               | +60               | +90              | +120             |
| 4 hours:    |                                      |                  |                  |                   |                   |                  |                  |
| ACTH        | 172.3 $\pm$ 27.0                     | 213.3 $\pm$ 46.5 | 242.0 $\pm$ 57.4 | 250.5 $\pm$ 55.1  | 252.8 $\pm$ 46.1  | 249.0 $\pm$ 46.7 | 251.0 $\pm$ 50.6 |
| 24 hours:   |                                      |                  |                  |                   |                   |                  |                  |
| ACTH        | 126.8 $\pm$ 32.1                     | 239.4 $\pm$ 61.9 | 244.4 $\pm$ 61.9 | 357.2 $\pm$ 112.0 | 439.4 $\pm$ 141.1 | 358.2 $\pm$ 99   | 159.8 $\pm$ 0.2  |
| Saline      | 87.4 $\pm$ 15.5                      | 60.1 $\pm$ 30.5  | 61.2 $\pm$ 21.6  | 59.2 $\pm$ 20.9   | 82.2 $\pm$ 21.1   | 60.3 $\pm$ 22.6  | 67.3 $\pm$ 16.7  |
| 1 week:     |                                      |                  |                  |                   |                   |                  |                  |
| ACTH        | 29.1 $\pm$ 6.1                       | 118.8 $\pm$ 23.8 | 184.2 $\pm$ 63.5 | 165.5 $\pm$ 39.2  | 199.4 $\pm$ 72.4  | 200.1 $\pm$ 71.8 | 61.3 $\pm$ 30.7  |
| Saline      | 50.0 $\pm$ 11.9                      | 62.6 $\pm$ 23.4  | 62.9 $\pm$ 18.5  | 57 $\pm$ 27.4     | 68.7 $\pm$ 34.3   | 64.25 $\pm$ 16.2 | 79.4 $\pm$ 26.5  |
| 1-2 Months: |                                      |                  |                  |                   |                   |                  |                  |
| ACTH        | 17.6 $\pm$ 3.1                       | 76.7 $\pm$ 8.3   | 92.5 $\pm$ 8.9   | 104.1 $\pm$ 8.8   | 107.4 $\pm$ 9.9   | 75.4 $\pm$ 6.9   | 50.0 $\pm$ 7.8   |
| Saline      | 14.9 $\pm$ 3.1                       | 22.7 $\pm$ 3.7   | 12.6 $\pm$ 2.0   | 16.7 $\pm$ 3.7    | 21.6 $\pm$ 7.7    | 18.6 $\pm$ 12.0  | 16.0 $\pm$ 15    |
| Adult:      |                                      |                  |                  |                   |                   |                  |                  |
| ACTH        | 28.9 $\pm$ 7.0                       | 123.2 $\pm$ 21.9 | 118.2 $\pm$ 33.4 | 143.4 $\pm$ 25.4  | 143.8 $\pm$ 32.8  | 108.0 $\pm$ 24.6 | 62.8 $\pm$ 14.6  |

injections. An additional sample at +75 minutes was taken after the 60-minute infusion studies and sampling was continued until 150 minutes with combined  $\alpha$ -MSH or ACTH and PGE<sub>2</sub> infusion studies. Samples were collected into chilled heparinized tubes, then centrifuged at 4° C. Plasma was stored at -20° C until analysis.

**Analysis.** Data were statistically evaluated by means of the t test for comparisons.

**Radioimmunoassay.** Cortisol was measured by a radioimmunoassay which has been previously described and evaluated.<sup>9</sup>

**Results**

The three basal cortisol concentrations in each of the experimental groups were not significantly different

and have been combined for presentation. The PGE<sub>2</sub> bolus injection into the brachiocephalic trunk significantly raised plasma cortisol above basal concentrations (Table I, P < 0.001). The 50  $\mu$ g bolus injection of PGE<sub>2</sub> into the distal thoracic aorta also significantly (P < 0.001) elevated cortisol above basal levels. Infusion of PGE<sub>2</sub> at 1.25  $\mu$ g/min for 15 minutes produced a rise in cortisol of comparable magnitude to that produced by the bolus injections. The rise was sustained for the duration of the 60-minute infusion so that the concentration of cortisol is significantly greater at 60 minutes in the long infusion than after intra-aortic injection (P < 0.05, Table I).

Basal plasma cortisol concentrations decreased from 34.3  $\pm$  7.3 ng/ml in the 9- to 11-day group to less than 19 ng/ml in the two older groups, as shown in Fig. 1.

**Table III.** Cortisol concentrations after ACTH and  $\alpha$ -MSH with and without PGE<sub>2</sub>

|                                                                        | Plasma cortisol (ng/ml) ( $\pm$ SEM) |                 |                 |                  |                 |
|------------------------------------------------------------------------|--------------------------------------|-----------------|-----------------|------------------|-----------------|
|                                                                        | Mean basal                           | +15             | +30             | +45              | +60             |
| <i>After ACTH with and without PGE<sub>2</sub>:</i>                    |                                      |                 |                 |                  |                 |
| ACTH alone (n = 4)                                                     | 10.7 $\pm$ 0.9                       | 15.4 $\pm$ 5.0  | 18.7 $\pm$ 3.5  | 33.4 $\pm$ 6.7   | 44.7 $\pm$ 11.6 |
| ACTH + PGE <sub>2</sub> (n = 3)                                        | 6.0 $\pm$ 0.7                        | 13 $\pm$ 4.4    | 18.6 $\pm$ 6.1  | 27.6 $\pm$ 2.6   | 33.1 $\pm$ 1.6  |
| <i>After <math>\alpha</math>-MSH with and without PGE<sub>2</sub>:</i> |                                      |                 |                 |                  |                 |
| $\alpha$ -MSH alone (n = 4)                                            | 20.5 $\pm$ 5.3                       | 67.6 $\pm$ 21.4 | 51.9 $\pm$ 15.0 | 64.4 $\pm$ 34.3  | 30.8 $\pm$ 12.9 |
| $\alpha$ -MSH + PGE <sub>2</sub> (n = 3)                               | 10.5 $\pm$ 3.9                       | 51.7 $\pm$ 8.1  | 96.1 $\pm$ 6.7  | 101.7 $\pm$ 12.4 | 90.4 $\pm$ 17.7 |

Infusion is from time 0 to +60 minutes of PGE<sub>2</sub> at 1.25  $\mu$ g/ml. Injection at time +30 minutes of 10  $\mu$ g of ACTH or 100  $\mu$ g of  $\alpha$ -MSH.

Peak cortisol concentrations achieved during a 60-minute PGE<sub>2</sub> infusion were significantly greater ( $P < 0.01$ ) in the youngest group than at the two later times.

A similar effect of advancing postnatal age is seen upon the cortisol response to ACTH stimulation in newborn lambs. As shown in Table II, the basal level falls and the response to stimulation increases between 4 and 24 hours. Subsequently both the basal concentration and the peak concentration after ACTH are less at 1 week and 1 to 2 months and in the adult.

Cortisol concentrations were significantly increased (Table III) after ACTH or  $\alpha$ -MSH bolus injections. The administration of ACTH or  $\alpha$ -MSH during PGE<sub>2</sub> infusion did not further increase the plasma cortisol concentrations above that elevation which had already been achieved by the PGE<sub>2</sub> infusion itself.

#### Comment

The injection of PGE<sub>2</sub> into the brachiocephalic trunk produces a rise in cortisol that is probably a direct action upon the brain or pituitary because metabolism of prostaglandin in the lung<sup>10</sup> should almost entirely eliminate the administered prostaglandin. This finding reproduces our earlier demonstration of an increase in cortisol in response to a brachiocephalic bolus injection of PGE<sub>2</sub>.<sup>8</sup> In that study the injection of PGE<sub>2</sub> into the distal aorta produced no increase in cortisol. This is in contrast to the present work and suggests that in the previous study not only was the injection probably not delivered to the adrenals in sufficient amounts but also there was a sufficient prostaglandin-degrading capability, presumably in the lung, that the central effect which did follow PGE<sub>2</sub> injection into the brachiocephalic trunk was eliminated. Earlier studies demonstrated that cortisol increased following intracarotid infusion of PGE<sub>2</sub> to the fetal sheep.<sup>7</sup> This observation has been confirmed in studies which also demonstrated that ACTH was released during an infusion of PGE<sub>2</sub> into the fetus.<sup>11</sup> However, the rise in cortisol in the fetus occurred at a time in gestation when the adre-

nal responds poorly to exogenous<sup>12</sup> or endogenous<sup>4</sup> ACTH. Our work has shown no augmentation of the PGE<sub>2</sub> response with concurrent ACTH administration, which suggests that the cortisol rise was not a synergistic effect occurring in the fetus. The fetal situation is complicated by the fact that the extensive shunting of blood and the very low percentage of cardiac output to the lung makes it probable that a prostaglandin injected anywhere in the fetal circulation may go everywhere in the fetus. Thus, an effect upon the brain/pituitary and/or directly upon the adrenal remain possibilities. Nevertheless, the present work suggests that in the newborn lamb there is an effect of prostaglandins upon the brain/pituitary which results in a cortisol increase. This effect must involve the secretion of adrenal trophic factor(s), possibly other than ACTH. Stimulation of pituitary hormone secretion by prostaglandin has been demonstrated.<sup>13</sup> An increase in ACTH following highly selective injection of prostaglandins into the medial basal hypothalamus in rats suggests an effect upon corticotropin-releasing factor.<sup>13</sup>

A direct effect upon the adrenal is suggested by the response to bolus injection and infusions into the distal thoracic aorta. The contrast with our previous work suggests that on that occasion, perhaps because of streaming of the blood flow, our bolus injections failed to reach the adrenal gland. In vitro studies in the adult have demonstrated elevation of cyclic adenosine monophosphate and stimulation of steroidogenesis by the adrenal in response to prostaglandins.<sup>14</sup> Very low doses of PGE<sub>2</sub>, as shown by the response to infusions of 1.25  $\mu$ g/min for only 15 minutes, are sufficient to elicit a response equal to that of a considerably larger bolus injection. A decrease in basal cortisol levels with advancing postnatal age may be due in part to a decrease in cortisol-binding globulin. However, the marked reduction in the response of the gland to PGE<sub>2</sub> infusions suggests a decreased sensitivity of the gland to stimulation or decreased capability of steroid synthesis. The response to ACTH is slight at 4 hours of age when the gland has already secreted nearly maximally. The sub-



| +75         | +90         | +120        | +150        |
|-------------|-------------|-------------|-------------|
| 39.7 ± 9.6  | 34.7 ± 11.8 | 14.6 ± 6.8  | 8.6 ± 0.8   |
| 31.2 ± 1.7  | 36.2 ± 6.2  | 10.4 ± 0.8  | 5.0 ± 0.9   |
| 24.2 ± 12.3 | 23.1 ± 8.6  | 42.9 ± 16.5 | 28.0 ± 15.3 |
| 51.4 ± 7.4  | 18.3 ± 5.3  | 11.1 ± 8.5  | 17.2 ± 15.7 |

sequent decrease in response at 7 and 28 days suggests diminished adrenal sensitivity with increase in length of time after delivery.

Both ACTH and  $\alpha$ -MSH are capable of raising cortisol concentrations in lambs. Failure of either of these substances to raise cortisol concentration further during a PGE<sub>2</sub> infusion suggests that the gland may already have been secreting maximally under the influence of PGE<sub>2</sub>. The similarity of cortisol concentrations obtained with a 15-minute infusion of 1.25  $\mu$ g/min of PGE<sub>2</sub> compared to a 50  $\mu$ g bolus injection of

PGE<sub>2</sub> suggests that low doses of prostaglandin may achieve maximum stimulation. The data are consistent with a role of PGE<sub>2</sub> as an intermediate factor in the action of the peptide hormones upon the adrenal gland.

Two sites of action of PGE<sub>2</sub> in the lamb, which result in an increase in cortisol secretion by different mechanisms, have been demonstrated in these experiments. The decline in cortisol secretory response with advancing gestational age is consistent with a falling away of the adrenal gland from a state of peripartum activation. The concentration of prostaglandins, particularly PGE<sub>2</sub>, rises in the fetus as term approaches.<sup>6</sup> The combination of increased sensitivity of the adrenal gland to several trophic factors and the availability of these factors, some of which may be unique to the fetal situation, in late gestation then allow the activation of the adrenal gland and a burst of cortisol secretion, which both prepares the lamb for extrauterine life and initiates parturition.

Supply of the PGE<sub>2</sub> used in this study by The Upjohn Co. of Canada, Don Mills, Ontario, is acknowledged.

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## ITEMS

### Medical meetings—1981

1. Third World Congress of Human Reproduction, March 22-26, 1981, Berlin, West Germany
2. The Fifth World Congress of Sexology June 21-26, 1981, Jerusalem, Israel
3. Second International Congress of Andrology, June 28-30, 1981, Tel Aviv, Israel
4. Second International Dermatopathology Symposium, July 12-15, 1981, London, England
5. The Sixth International Congress of Human Genetics, September 13-18, 1981, Jerusalem, Israel

For a brochure please write or call: Medical Congress Coordinators, 1212 Avenue of the Americas, New York, New York 10036. Telephone: (212) 840-3110.

### Fourth Annual Meeting of the American College of Cryosurgery

The American College of Cryosurgery announces a call for abstracts for their Fourth Annual Meeting, on Friday through Sunday, June 12-14, 1981, at the Marriott Crystal City in Arlington, Virginia.

For further information on abstracts or the meeting, contact: Daniel Moran, 18 West Main St., Clinton, Connecticut 06413.

### Review Course in Neonatology/Perinatology

A Review Course in Neonatology/Perinatology will be presented August 31-September 4, 1981, sponsored by The Cook County Graduate School of Medicine.

For further information and registration contact: The Cook County Graduate School of Medicine, 707 South Wood St., Chicago, Illinois 60612.

### Sixth Annual Conference on Clinical Application of Hyperbaric Oxygen

The Sixth Annual Conference on Clinical Application of Hyperbaric Oxygen is scheduled for June 10-12, 1981, at the Memorial Hospital Medical Center of Long Beach—University of California, Irvine Center for Health Education.

For further information contact: G. B. Hart, M.D., Director, Baromedical Department, Memorial Hospital Medical Center, 2801 Atlantic Ave., Long Beach, California 90801.

### Third World Congress of Human Reproduction

The Third World Congress of Human Reproduction will be held in Berlin, West Germany, March 22-26, 1981.

For scientific and travel information, contact the officially appointed agent for the United States: Medical Congress Coordinators, 1212 Avenue of the Americas, New York, New York 10036.

### Cancer Prevention and Detection Modules for Nurses, 1981

"Cancer Prevention and Detection Modules for Nurses, 1981" will be presented by The University of Texas System Cancer Center, M. D. Anderson Hospital and Tumor Institute, Houston, Texas.

For the schedule and information, contact: Judy E. Patterson, R.N., Cancer Prevention and Detection Program for Nurses, M. D. Anderson Hospital, 6723 Bertner Ave., Room PR855, Houston, Texas 77030.

### Health Issues for Older Women: A Projection for the Year 2000

The School of Allied Health Professions, State University of New York at Stony Brook, and the National Action Forum for Older Women will present an Invitational Conference, entitled, "Health Issues for Older Women: A Projection for the Year 2000," at the Health Sciences Center, State University of New York at Stony Brook on April 1, 2, and 3, 1981.

For further information contact: Peggy Bruhn, Conference Coordinator, School of Allied Health Professions, HSC, SUNY/SB, Stony Brook, New York 11794.

### First International Symposium on Minimal Invasive Cancer

The First International Symposium on Minimal Invasive Cancer, organized by the University Clinic of Obstetrics and Gynecology, Graz, will be held at Graz, Kongresshaus, July 1-4, 1981. The main topics are: Cervix, Vulva, Endometrium, Breast, Esophagus, Stomach, Large intestine, Lung, Thyroid, Urinary bladder, Prostate, Larynx, Oral cavity, Skin, and Other Organs. The Congress language is English.

For information write the Secretariat: Interconvention, P. O. Box 105 A-1014 Vienna, Austria.



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1.



2.



3.



4.

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2. She wants nonhormonal contraception.
3. She smokes.
4. She is over 35.

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**Contraindications:** Not to be inserted when any of the following conditions exist: pregnancy or suspicion of pregnancy; abnormalities of the uterus resulting in distortion of the uterine cavity; acute pelvic inflammatory disease or history of repeated pelvic inflammatory disease; postpartum endometritis or infected abortion in the past three months; known or suspected uterine or cervical disease such as hyperplasia or carcinoma including unresolved, abnormal "Pap" smear; vaginal bleeding of unknown etiology; untreated acute cervicitis until infection is controlled; diagnosed Wilson's disease; known or suspected allergy to copper; previous ectopic pregnancy; significant anemia; and valvular heart disease, leukemia, or use of chronic corticosteroid therapy.

## Warnings

1. **Pregnancy:** (a) **Long-term effects.** The long-term effects on the offspring of the presence of copper in the uterus when pregnancy occurs are unknown.

(b) **Septic abortion.** Reports have indicated an increased incidence of septic abortion associated in some instances with septicemia, septic shock and death in patients becoming pregnant with an IUD in place. Most of these reports have been associated with the mid-trimester of pregnancy. In some cases, the initial symptoms have been insidious and not easily recognized. If pregnancy should occur with a Cu-7 or Tatum-T in situ, the IUD should be removed if the thread is visible or, if removal proves to be or would be difficult, interruption of the pregnancy should be considered and offered to the patient as an option, bearing in mind that the risks associated with an elective abortion increase with gestational age.

(c) **Continuation of pregnancy.** If the patient chooses to continue the pregnancy and the Cu-7 or Tatum-T remains in situ, she must be warned of increased risk of spontaneous abortion and increased risk of sepsis, including death. The patient must be closely observed and advised to report immediately all abnormal symptoms, such as flu-like syndrome, fever, abdominal cramping and pain, bleeding, or vaginal discharge, because generalized symptoms of septicemia may be insidious.

2. **Ectopic pregnancy:** (a) Pregnancy which occurs with an IUD in situ is more likely to be ectopic than pregnancy occurring without an IUD. Therefore, patients who become pregnant while using a Cu-7 or Tatum-T should be carefully evaluated for the possibility of ectopic pregnancy.

(b) Special attention should be directed to patients with delayed menses, slight metrorrhagia and/or unilateral pelvic pain, and to those patients who wish to interrupt a pregnancy occurring in the presence of a Cu-7 or Tatum-T, to determine whether ectopic pregnancy has occurred.

3. **Pelvic infection:** An increased risk of pelvic inflammatory disease associated with the use of IUDs has been reported. While unconfirmed, this risk appears to be greatest for young women who are nulliparous and/or who have multiple sexual partners. Salpingitis can result in tubal damage and occlusion, thereby threatening future fertility. Therefore, it is recommended that patients be taught to look for symptoms of pelvic inflammatory disease. The decision to use an IUD in a particular case must be made by the physician and patient with consideration of a possible deleterious effect on future fertility.

Pelvic infection may occur with a Cu-7 or Tatum-T in situ and at times result in the development of tubo-ovarian abscesses or general peritonitis. The symptoms of pelvic infection include: new development of menstrual disorders (prolonged or heavy bleeding), abnormal vaginal discharge, abdominal or pelvic pain, dyspareunia, fever. The symptoms are especially significant if they occur following the first few cycles after insertion. Appropriate aerobic and anaerobic bacteriologic studies should be done and antibiotic therapy initiated promptly. If the infection does not show marked clinical improvement within 24 to 48 hours, the Cu-7 or Tatum-T should be removed and continuing treatment reassessed on the basis of results of culture and sensitivity tests.

4. **Embedment:** Partial penetration or lodging of the Cu-7 or Tatum-T in the endometrium or myometrium can result in difficult removal. This may occur more frequently in smaller uteri. See removal instructions.

5. **Perforation:** Partial or total perforation of the uterine wall or cervix may occur, usually during insertions into patients sooner than two months after abortion or delivery, or in uterine cavities too small. The possibility of perforation must be kept in mind during insertion and at the time of any subsequent examination. If perforation occurs, laparotomy or laparoscopy should be performed as soon as medically feasible and the Cu-7 or Tatum-T removed. Abdominal adhesions, intestinal penetration, intestinal obstruction, and local inflammatory reaction with abscess formation and erosion of adjacent viscera may result if the Cu-7 or Tatum-T is left in the peritoneal cavity.

6. **Medical diathermy:** The use of medical diathermy (short-wave and microwave) in a patient with a metal-containing IUD may cause heat injury to surrounding tissue. Therefore, medical diathermy to the abdominal and sacral areas should not be used on patients using a Cu-7 or Tatum-T.

7. **Effects of copper:** Additional amounts of copper available to the body from the Cu-7 or Tatum-T may precipitate symptoms in women with undiagnosed Wilson's disease. The incidence of Wilson's disease is 1 in 20,000.

## Precautions

1. **Patient counseling.** Prior to insertion the physician, nurse, or other trained health professional must provide the patient with the Patient Brochure. The patient should be given the opportunity to read the brochure and discuss fully any questions she may have concerning the Cu-7 or Tatum-T as well as other methods of contraception.

2. **Patient evaluation and clinical considerations.** (a) A complete medical history should be obtained to determine conditions that might influence the selection of an IUD. A physical examination should include a pelvic examination, "Pap" smear, gonorrhea culture and, if indicated, appropriate tests for other forms of venereal disease. The physician should determine that the patient is not pregnant.

(b) The uterus should be carefully sounded prior to insertion to determine the degree of patency of the endocervical canal and internal os, and the direction and depth of the uterine

cavity. Exercise care to avoid perforation with the sound. DO NOT USE THE INSERTION INSTRUMENT AS A SOUND. In occasional cases, severe cervical stenosis may be encountered. Do not use excessive force to overcome this resistance.

(c) The uterus usually sounds to a depth of 6 to 8 cm. Insertion into a uterine cavity measuring less than 6.5 cm. by sounding may increase the incidence of pain, bleeding, partial or complete expulsion, perforation, and possibly pregnancy.

(d) To reduce the possibility of insertion in the presence of existing undetermined pregnancy, the optimal time for insertion is the latter part of the menstrual flow or one or two days thereafter. The Cu-7 or Tatum-T should not be inserted post partum or post abortion until involution of the uterus is complete. The incidence of perforation and expulsion is greater if involution is not complete.

It is, however, necessary to place the Cu-7 or Tatum-T as high as possible within the uterine cavity to help avoid partial or complete expulsion that could result in pregnancy.

Physicians are cautioned that it is imperative for them to become thoroughly familiar with the instructions for use before attempting placement of the Cu-7 or Tatum-T.

(e) IUDs should be used with caution in those patients who have anemia or a history of menorrhagia or hypermenorrhea. Patients experiencing menorrhagia and/or metrorrhagia following IUD insertion may be at risk for the development of hypochromic microcytic anemia. Also, IUDs should be used with caution in patients receiving anticoagulants or having a coagulopathy.

(f) Syncope, bradycardia or other neurovascular episodes may occur during insertion or removal of IUDs, especially in patients with a previous disposition to these conditions.

(g) Patients with valvular or congenital heart disease are more prone to develop subacute bacterial endocarditis than patients who do not have such disease. Use of an IUD in these patients may represent a potential source of septic emboli.

(h) Use of an IUD in patients with acute cervicitis should be postponed until proper treatment has cleared up the infection.

(i) Since the Cu-7 or Tatum-T may be partially or completely expelled, patients should be reexamined and evaluated shortly after the first postinsertion menses, but definitely within three months after insertion. Thereafter annual examination with appropriate medical and laboratory evaluation should be carried out. The Cu-7 or Tatum-T should be replaced every three years.

(j) The patient should be told that some bleeding or cramping may occur during the first few weeks after insertion, but if these symptoms continue or are severe she should report them to her physician. She should be instructed on how to check after each menstrual period to make certain that the thread still protrudes from the cervix and cautioned that there is no contraceptive protection if the Cu-7 or Tatum-T has been expelled. She should also be cautioned not to dislodge the Cu-7 or Tatum-T by pulling on the thread. If a partial expulsion occurs, removal is indicated and a new Cu-7 or Tatum-T may be inserted. The patient should be told to return within three years for removal of the Cu-7 or Tatum-T and for replacement if desired.

(k) A copper-induced urticarial allergic skin reaction may develop in women using a copper-containing IUD. If symptoms of such an allergic response occur, the patient should be instructed to tell the consulting physician that a copper-containing device is being used.

(l) The Cu-7 or Tatum-T should be removed for the following medical reasons: menorrhagia and/or metrorrhagia producing significant anemia; uncontrolled pelvic infection; intractable pain often aggravated by intercourse, dyspareunia; pregnancy, if the thread is visible; endometrial or cervical malignancy; uterine or cervical perforation; or any indication of partial expulsion.

(m) If the retrieval thread cannot be seen it may have retracted into the uterus or have been broken off, or the Cu-7 or Tatum-T may have been expelled. Localization usually can be made by feeling with a probe; if not, x-ray or sonography can be used. When the physician elects to recover a Cu-7 or Tatum-T with the thread not visible, the removal instructions should be considered.

(n) If any patient with a Cu-7 or Tatum-T suddenly develops overt clinical hepatitis or abnormal liver function tests, appropriate diagnostic procedures should be initiated.

**Adverse Reactions:** Perforations of uterus and cervix have occurred. Perforation into the abdomen has been followed by abdominal adhesions, intestinal penetration, intestinal obstruction, local inflammatory reaction with abscess formation and erosion of adjacent viscera. Pregnancy has occurred with the Cu-7 or Tatum-T in situ and when either has been partially or completely expelled.

The incidence of spontaneous abortion, when conception occurs with intrauterine devices in situ, appears to be increased over that in unprotected women. Insertion cramping, usually of no more than a few seconds' duration, may occur; however, some women may experience residual cramping for several hours or even days. Intermenstrual spotting or bleeding or prolonged or increased menstrual flow may occur.

Pelvic infection including salpingitis with tubal damage or occlusion has been reported. This may result in future infertility. Complete or partial expulsion of the Cu-7 or Tatum-T may sometimes occur, particularly in those patients with uteri measuring less than 6.5 cm. by sounding. Urticarial allergic skin reaction may occur. The following complaints have also been reported with IUDs although their relation to the Cu-7 or Tatum-T has not been established: amenorrhea or delayed menses, backaches, cervical erosion, cystic masses in the pelvis, vaginitis, leg pain or soreness, weight loss or gain, nervousness, dyspareunia, cystitis, endometritis, septic abortion, septicemia, leukorrhea, ectopic pregnancy, difficult removal, uterine embedment, anemia, pain, neurovascular episodes including bradycardia and syncope secondary to insertion, dysmenorrhea, and fragmentation of the IUD.

**Clinical Efficacy:** In clinical trials, use effectiveness was determined as follows for parous and nulliparous women, as tabulated by the life table method. (Rates are expressed as cumulative events per 100 women through 12, 24, and 36 months of use.)

Cu-7 experience encompasses 387,131 woman-months of use, including 12 months for 11,517 women, 24 months for 7,895, and 36 months for 3,661.

Tatum-T experience encompasses 236,060 woman-months of use in the United States and Canada, including 12 months for 8,232 women, 24 months for 4,247, and 36 months for 1,408.

Cumulative pregnancy rates were:

|         | 12 Months |             | 24 Months |             | 36 Months |             |
|---------|-----------|-------------|-----------|-------------|-----------|-------------|
|         | Parous    | Nulliparous | Parous    | Nulliparous | Parous    | Nulliparous |
| Cu-7    | 1.9       | 1.7         | 2.9       | 2.6         | 3.4       | 3.4         |
| Tatum-T | 3.0       | 2.1         | 4.9       | 4.5         | 6.0       | 5.8         |

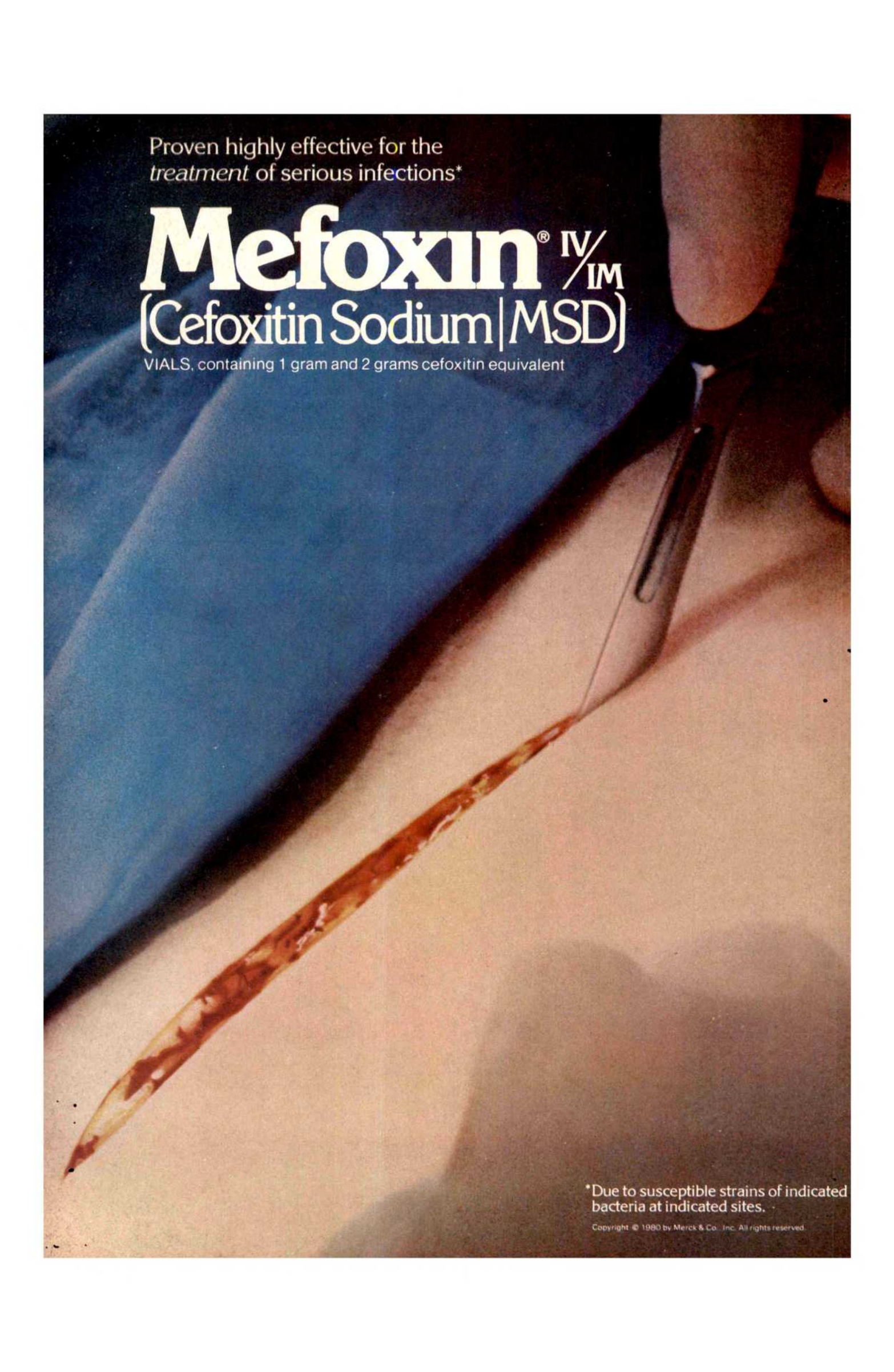
Please see complete product labeling for additional information, including insertion and removal instructions.

**SEARLE**

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071-2





Proven highly effective for the  
*treatment* of serious infections\*

# Mefoxin<sup>®</sup> IV/IM

(Cefoxitin Sodium | MSD)

VIALS, containing 1 gram and 2 grams cefoxitin equivalent

\*Due to susceptible strains of indicated  
bacteria at indicated sites.

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# Now Indicated for prophylaxis to reduce the incidence of certain postoperative infections complicating

• GI surgery<sup>†</sup> • Vaginal hysterectomy<sup>†</sup> • Cesarean section<sup>‡</sup>

Providing a broad spectrum—including *Bacteroides fragilis*

In controlled clinical trials, MEFOXIN<sup>®</sup> (Cefoxitin Sodium, MSD)  
reduced the incidence of certain postoperative infections  
with use limited to a 24-hour period following the operative procedure.

Prophylactic administration should usually be stopped within 24 hours since continuing administration of any antibiotic increases the possibility of adverse reactions.

**†Perioperatively:** Two grams administered intravenously or intramuscularly just prior to surgery (approximately ½ to 1 hour before the initial incision); then 2 grams every 6 hours for no more than 24 hours.

**‡Cesarean-section patients:** The first dose of 2 grams is administered intravenously as soon as the umbilical cord is clamped. The second and third doses should be given as 2 grams intravenously or intramuscularly 4 hours and 8 hours after the first dose. Subsequent doses may be given every 6 hours for no more than 24 hours.

For complete details on dosage and administration, see full prescribing information.

If there are signs of infection, specimens for culture should be obtained for the identification

of the causative organisms so that appropriate therapy may be instituted.

MEFOXIN (Cefoxitin Sodium, MSD) is contraindicated in patients who have shown hypersensitivity to cefoxitin and the cephalosporin group of antibiotics. Before therapy with MEFOXIN is instituted, careful inquiry should be made to determine whether the patient has had previous hypersensitivity reactions to cefoxitin, cephalosporins, penicillins, or other drugs. This product should be given with caution to penicillin-sensitive patients. Antibiotics should be administered with caution to any patient who has demonstrated some form of allergy, particularly to drugs. If an allergic reaction to MEFOXIN occurs, discontinue the drug. Serious hypersensitivity reactions may require epinephrine and other emergency measures. Use of the drug in women of childbearing potential requires that the anticipated benefit be weighed against the possible risks.

For a brief summary of prescribing information, please see following page.

**MSD**  
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DOHME



# Mefoxin<sup>®</sup> IV/IM

(Cefoxitin Sodium | MSD)

**Indications and Usage: Treatment**—Serious infections caused by susceptible strains of the designated microorganisms in the following diseases:

**LOWER RESPIRATORY TRACT INFECTIONS**, including pneumonia and lung abscess, caused by *Streptococcus pneumoniae* (formerly *Diplococcus pneumoniae*), other streptococci (excluding enterococci, e.g., *Strep. faecalis*), *Staphylococcus aureus* (penicillinase and non-penicillinase producing), *Escherichia coli*, *Klebsiella* species, *Hemophilus influenzae*, and *Bacteroides* species.

**GENITOURINARY INFECTIONS**. Urinary tract infections caused by *E. coli*, *Klebsiella* species, *Proteus mirabilis*, indole-positive *Proteus* (i.e., *P. morganii*, *P. rettgeri*, and *P. vulgaris*), and *Providencia* species. Uncomplicated gonorrhea due to *Neisseria gonorrhoeae*.

**INTRA-ABDOMINAL INFECTIONS**, including peritonitis and intra-abdominal abscess, caused by *E. coli*, *Klebsiella* species, *Bacteroides* species including the *B. fragilis* group,<sup>‡</sup> and *Clostridium* species. **GYNECOLOGICAL INFECTIONS**, including endometritis, pelvic cellulitis, and pelvic inflammatory disease, caused by *E. coli*, *N. gonorrhoeae*, *Bacteroides* species including the *B. fragilis* group,<sup>‡</sup> *Clostridium* species, *Peptococcus* species, *Peptostreptococcus* species, and group B streptococci.

**SEPTICEMIA** caused by *Strep. pneumoniae* (formerly *D. pneumoniae*), *Staph. aureus* (penicillinase and non-penicillinase producing), *E. coli*, *Klebsiella* species, and *Bacteroides* species including the *B. fragilis* group.<sup>‡</sup>

**BONE AND JOINT INFECTIONS** caused by *Staph. aureus* (penicillinase and non-penicillinase producing).

**SKIN AND SKIN STRUCTURE INFECTIONS** caused by *Staph. aureus* (penicillinase and non-penicillinase producing), *Staph. epidermidis*, streptococci (excluding enterococci, e.g., *Strep. faecalis*), *E. coli*, *P. mirabilis*, *Klebsiella* species, *Bacteroides* species including the *B. fragilis* group,<sup>‡</sup> *Clostridium* species, *Peptococcus* species, and *Peptostreptococcus* species.

Although appropriate culture and susceptibility studies should be performed, therapy may be started while awaiting these results. Cefoxitin is not active *in vitro* against most strains of *Pseudomonas aeruginosa* and enterococci (e.g., *Strep. faecalis*) and many strains of *Enterobacter cloacae*. Methicillin-resistant staphylococci are almost uniformly resistant to cefoxitin.

**Prevention**—Prophylactic use perioperatively (preoperatively, intraoperatively, and postoperatively) in surgical procedures (e.g., vaginal hysterectomy, gastrointestinal surgery) classified as contaminated or potentially contaminated or in patients in whom infection at the operative site would present a serious risk, e.g., prosthetic arthroplasty; intraoperatively (after umbilical cord is clamped) and postoperatively in cesarean section.

MEFOXIN usually should be given ½ to 1 hour before the operation, which is sufficient time to achieve effective levels in the wound during the procedure. Prophylactic administration should usually be stopped within 24 hours since continuing administration of any antibiotic increases the possibility of adverse reactions but, in the majority of surgical procedures, does not reduce the incidence of subsequent infection. However, in patients undergoing prosthetic arthroplasty, it is recommended that MEFOXIN be continued for 72 hours after the surgical procedure. If there are signs of infection, specimens for culture should be obtained for the identification of the causative organism so that appropriate therapy may be instituted.

**Contraindications:** Previous hypersensitivity to cefoxitin and the cephalosporin group of antibiotics.

**Warnings:** BEFORE THERAPY IS INSTITUTED, CAREFUL INQUIRY SHOULD BE MADE TO DETERMINE PREVIOUS HYPERSENSITIVITY REACTIONS TO CEFOXITIN, CEPHALOSPORINS, PENICILLINS, OR

OTHER DRUGS. GIVE WITH CAUTION TO PENICILLIN-SENSITIVE PATIENTS. ANTIBIOTICS SHOULD BE ADMINISTERED WITH CAUTION TO ANY PATIENT WHO HAS DEMONSTRATED SOME FORM OF ALLERGY, PARTICULARLY TO DRUGS. IF AN ALLERGIC REACTION TO CEFOXITIN OCCURS, DISCONTINUE THE DRUG. SERIOUS HYPERSENSITIVITY REACTIONS MAY REQUIRE EPINEPHRINE AND OTHER EMERGENCY MEASURES.

**Precautions:** The total daily dose should be reduced in patients with transient or persistent reduction of urinary output due to renal insufficiency because high and prolonged serum antibiotic concentrations can occur in such individuals from usual doses. As with other antibiotics, prolonged use may result in overgrowth of nonsusceptible organisms; repeated evaluation of the patient's condition is essential. If superinfection occurs, take appropriate measures. Increased nephrotoxicity has been reported following concomitant administration of cephalosporins and aminoglycoside antibiotics.

**Interference with Laboratory Tests**—As with cephalothin, high concentrations (> 100 mcg/ml) may interfere with measurement of serum and urine creatinine levels by the Jaffé reaction and produce false increases of modest degree in creatinine levels reported; serum samples should not be analyzed for creatinine if withdrawn within 2 hours of cefoxitin administration. A false-positive reaction for glucose in urine has been observed with CLINITEST<sup>®</sup> reagent tablets.

**Pregnancy**—In women of childbearing potential, weigh anticipated benefit against possible risks.

**Nursing Mothers**—Cefoxitin is excreted in human milk in low concentrations.

**Infants and Children**—Safety and efficacy in infants from birth to three months have not yet been established. In children three months and older, higher doses have been associated with increased incidence of eosinophilia and elevated SGOT.

**Adverse Reactions:** The most common adverse reactions have been local reactions following intravenous or intramuscular injection. Other adverse reactions have been encountered infrequently. **Local Reactions**—Thrombophlebitis with intravenous administration; pain, induration, and tenderness after intramuscular injections. **Allergic Reactions**—Rash, pruritus, eosinophilia, fever, and other allergic reactions. **Gastrointestinal**—Nausea, vomiting, and diarrhea. **Blood**—Transient eosinophilia, leukopenia, neutropenia, and hemolytic anemia; a positive direct Coombs test may develop in some individuals, especially those with azotemia. **Liver Function**—Transient elevations in SGOT, SGPT, serum LDH, and serum alkaline phosphatase. **Renal Function**—Elevations in serum creatinine and/or blood urea nitrogen levels.

**Note:** In group A beta-hemolytic streptococcal infections, therapy should be maintained for at least 10 days to guard against the risk of rheumatic fever or glomerulonephritis. In staphylococcal and other infections involving a collection of pus, surgical drainage should be carried out where indicated. Intramuscular injections should be well within the body of a relatively large muscle such as the upper outer quadrant of the buttock (i.e., gluteus maximus); aspiration is necessary to avoid inadvertent injection into a blood vessel. The total daily dosage in infants and children should not exceed 12 grams.

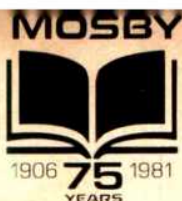
**How Supplied:** Sterile cefoxitin sodium in vials and infusion bottles containing 1 gram or 2 grams cefoxitin equivalent.

For more detailed information, consult your MSD representative or see full prescribing information, Merck Sharp & Dohme, Division of Merck & Co., Inc., West Point, PA 19486

<sup>‡</sup>*B. fragilis*, *B. distasonis*, *B. ovatus*, *B. thetaiotaomicron*, *B. vulgatus*  
\*Registered trademark of Ames Company, Division of Miles Laboratories, Inc.

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# American Journal of Obstetrics and Gynecology

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## Contents

February 15

1981

## Papers of the Society for Gynecologic Investigation

- Patterns of fetal lamb regional cerebral blood flow during and after prolonged hypoxia: Studies during the posthypoxic recovery period** 365

Stephen Ashwal, M.D., John S. Majcher, M.S., and Lawrence D. Longo, M.D.  
Loma Linda, California

- Initiation of human parturition. XII. Biosynthesis and metabolism of prostaglandins in human fetal membranes and uterine decidua** 373

Takeshi Okazaki, M.D., M. Linette Casey, B.S., Janice R. Okita, B.S.,  
Paul C. MacDonald, M.D., and John M. Johnston, Ph.D.  
Dallas, Texas

(Contents continued on page 7)

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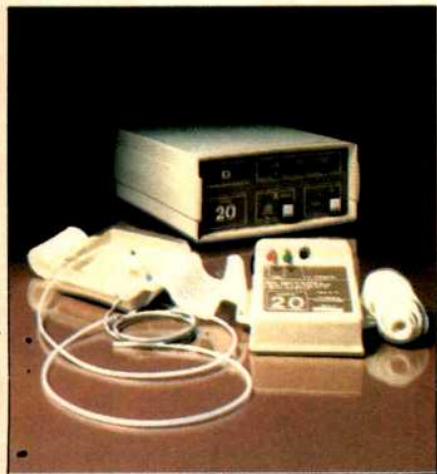
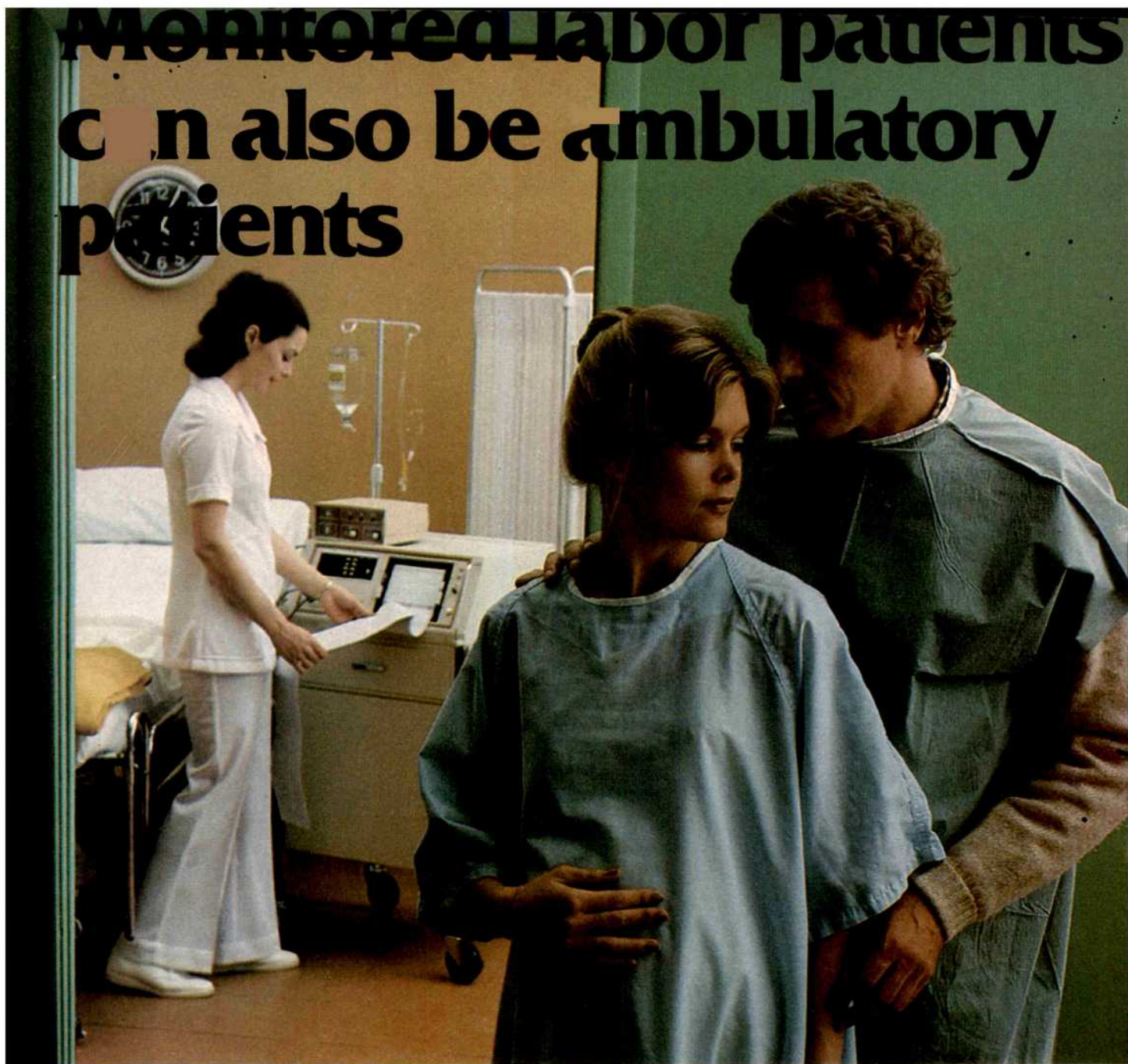
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\*Flynn, A.M., Kelly, J., Hollins, G., Lynch. Ambulation in Labor. *British Medical Journal*, 1978, 2, 591-593

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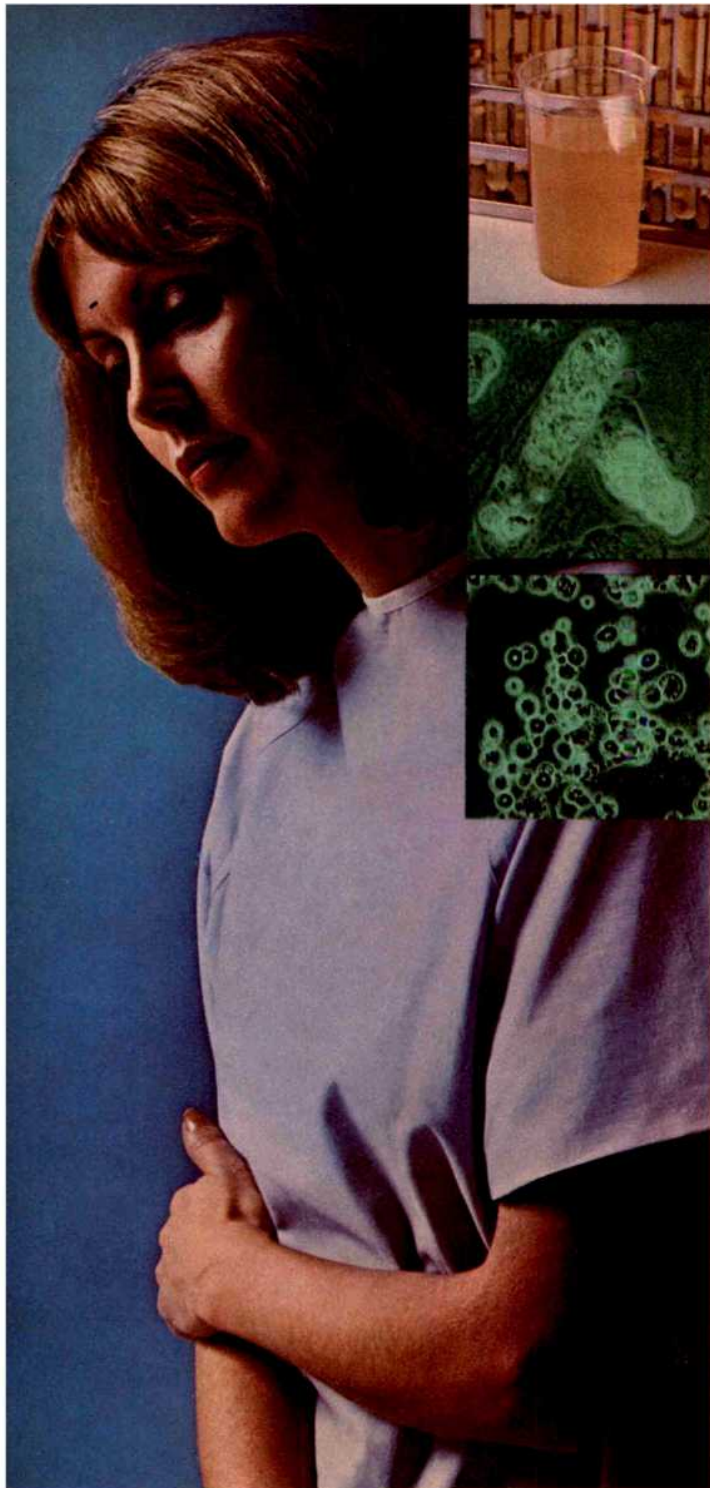


## Contents continued from page 5

|                                                                                                                                                                                                                                                                      |     |
|----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|-----|
| <b>Estriol in pregnancy. VII. Unconjugated plasma estriol in prolonged gestation</b>                                                                                                                                                                                 | 382 |
| Robert J. Gauthier, M.D., Bertram D. Griego, M.D., and Uwe Goebelsmann, M.D.<br><i>Los Angeles, California</i>                                                                                                                                                       |     |
| <b>Amniotic fluid C-peptide as an index for intrauterine fetal growth</b>                                                                                                                                                                                            | 390 |
| Chin-Chu Lin, M.D., Atef H. Moawad, M.D., Philip River, B.S., Petra Blix, B.S.,<br>Marilyn Abraham, B.S., and Arthur H. Rubenstein, M.D.<br><i>Chicago, Illinois</i>                                                                                                 |     |
| <b>Minor (glycosylated) hemoglobins in cord blood of infants of normal and diabetic mothers</b>                                                                                                                                                                      | 397 |
| Hossam E. Fadel, M.D., F.A.C.O.G., A. Reynolds, B.S., M. Stallings, B.S., and<br>E. C. Abraham, Ph.D.<br><i>Augusta, Georgia</i>                                                                                                                                     |     |
| <b>Bladder versus renal bacteriuria during pregnancy: Recurrence after treatment</b>                                                                                                                                                                                 | 403 |
| Kenneth J. Leveno, M.D., Robert E. Harris, M.D., Larry C. Gilstrap, M.D.,<br>Peggy J. Whalley, M.D., and F. Gary Cunningham, M.D., with the technical assistance of<br>Sheryl S. Kappus, M.S., and Brenda Nobles<br><i>Dallas and Lackland Air Force Base, Texas</i> |     |
| <b>The fetal and maternal catecholamine response to insulin-induced hypoglycemia in the rat</b>                                                                                                                                                                      | 407 |
| M. Phillippe, M.D., and J. L. Kitzmiller, M.D.<br><i>Boston, Massachusetts</i>                                                                                                                                                                                       |     |
| <b>Maternal plasma adrenocorticotropin and cortisol relationships throughout human pregnancy</b>                                                                                                                                                                     | 416 |
| Bruce R. Carr, C. Richard Parker, Jr., James D. Madden, Paul C. MacDonald, and<br>John C. Porter<br><i>Dallas, Texas</i>                                                                                                                                             |     |
| <b>The glucose tolerance test as a means of identifying intrauterine growth retardation</b>                                                                                                                                                                          | 423 |
| Victor A. Khouzami, M.D., David S. Ginsburg, M.D., Norman H. Daikoku, M.D., and<br>John W. C. Johnson, M.D.<br><i>Baltimore, Maryland</i>                                                                                                                            |     |
| <b>Lack of relationship between prolactin and adrenal steroidogenesis in the ovine fetus</b>                                                                                                                                                                         | 427 |
| Katsuhiko Takahashi, M.D., Frederick Auletta, Ph.D., Hsiu-Yu Tseng, M.S.,<br>Antonio Scommegna, M.D., and Laurence I. Burd, M.D.<br><i>Chicago, Illinois</i>                                                                                                         |     |

(Contents continued on page 9)





In acute UTI

When your first choice  
is Macrochantin®  
you'll rarely need  
a second one

**Macrochantin is potent against  
both major uropathogens**

in office practice—*E. coli*, the most common, and  
*enterococcus*—unlike other widely prescribed  
antimicrobials used individually or in combination.  
Macrochantin efficacy in vitro\* against *E. coli*,  
97%...against enterococcus, 94%.

**Superior in vitro\* against the two most common uropathogens†**

| <i>E. coli</i>          | <i>Enterococcus</i>     |
|-------------------------|-------------------------|
| <b>MACROCHANTIN 97%</b> | <b>MACROCHANTIN 94%</b> |
| <b>TMP/SMX 95%</b>      | <b>TMP/SMX 51%</b>      |
| <b>Sulfonamide 73%</b>  | <b>Sulfonamide 6%</b>   |

\*In vitro data do not necessarily predict clinical efficacy.

†. PMR Bacteriologic Report, Summer Series 1979; a national bacteriologic  
monitoring service for 200 acute-care hospitals of 100 beds or more.

...too valuable to keep in reserve

**Macrochantin®** Capsules:  
(nitrofurantoin macrocrystals) 25, 50, 100mg

**INDICATIONS:** Macrochantin is indicated for the treatment of urinary tract infections when due to susceptible strains of *E. coli*, enterococci, *S. aureus* (it is not indicated for the treatment of associated renal cortical or perinephric abscesses), and certain susceptible strains of *Klebsiella* species, *Enterobacter* species, and *Proteus* species.

**NOTE:** Specimens for culture and susceptibility testing should be obtained prior to and during drug administration.

**CONTRAINDICATIONS:** Anuria, oliguria, or significant impairment of renal function (creatinine clearance under 40 ml per minute) are contraindications. Treatment of this type of patient carries an increased risk of toxicity because of impaired excretion of the drug. For the same reason, this drug is much less effective under these circumstances.

Contraindicated in pregnant patients at term as well as in infants under one month of age because of the possibility of hemolytic anemia due to immature enzyme systems (glutathione instability), and those known to have a hypersensitivity to Macrochantin, Furadantin® (nitrofurantoin), and other nitrofurantoin preparations.

**WARNINGS:** Acute, subacute and chronic pulmonary reactions have been observed. If these reactions occur, the drug should be withdrawn and appropriate measures taken.

An insidious onset of pulmonary reactions (diffuse interstitial pneumonitis or pulmonary fibrosis, or both) in patients on long-term therapy warrants close monitoring.

There have been isolated reports giving pulmonary reactions as a contributing cause of death. (See Hypersensitivity reactions.)

Cases of hemolytic anemia of the primaquine sensitivity type have been induced. The hemolysis appears to be linked to a glucose-6-phosphate dehydrogenase deficiency in the red blood cells of the affected patients. This deficiency is found in 10 percent of Negroes and a small percentage of ethnic groups of Mediterranean and Near-Eastern origin. Any sign of hemolysis is an indication to discontinue the drug. Hemolysis ceases when the drug is withdrawn.

*Pseudomonas* is the organism most commonly implicated in superinfections in patients treated with Macrochantin.

Hepatitis, including chronic active hepatitis, has been observed rarely. Fatalities have been reported. The mechanism appears to be of an idiosyncratic hypersensitivity type.

**PRECAUTIONS:** Peripheral neuropathy may occur. This may become severe or irreversible. Fatalities have been reported. Predisposing conditions such as renal impairment (creatinine clearance under 40 ml per minute), anemia, diabetes, electrolyte imbalance, vitamin B deficiency, and debilitating disease may enhance such occurrence.

**Usage in Pregnancy:** Safety during pregnancy and lactation has not been established. Use of this drug in women of childbearing potential requires that the anticipated benefit be weighed against the possible risks.

**ADVERSE REACTIONS: Gastrointestinal reactions:** Anorexia, nausea and emesis are the most frequent reactions; abdominal pain and diarrhea occur less frequently. These dose-related toxicity reactions can be minimized by reduction of dosage, especially in the female patient. Hepatitis occurs rarely.

**Hypersensitivity reactions:** Pulmonary sensitivity reactions may occur, which can be acute, subacute, or chronic.

Acute reactions are commonly manifested by fever, chills, cough, chest pain, dyspnea, pulmonary infiltration with consolidation or pleural effusion on x-ray, and eosinophilia. They usually occur within the first week of treatment and are reversible with cessation of therapy. Resolution may be dramatic.

In subacute reactions, fever and eosinophilia are observed less often. Recovery is somewhat slower, perhaps as long as several months. If the symptoms are not recognized as being drug related and nitrofurantoin is not withdrawn, symptoms may become more severe.

Chronic pulmonary reactions are more likely to occur in patients who have been on continuous therapy for six months or longer. Insidious onset of malaise, dyspnea on exertion, cough, and altered pulmonary function are common manifestations. Roentgenographic and histologic findings of diffuse interstitial pneumonitis or fibrosis, or both, are also common manifestations. Fever is rarely prominent. The severity of these reactions and the degree of their resolution appear to be related to the duration of therapy after the first clinical signs appear. Pulmonary function may be permanently impaired even after cessation of nitrofurantoin therapy. This risk is greater when pulmonary reactions are not recognized early.

**Dermatologic reactions:** Maculopapular, erythematous, or eczematous eruption, pruritus, urticaria, and angioedema.

**Other hypersensitivity reactions:** Anaphylaxis, asthmatic attack in patients with history of asthma, cholestatic jaundice, hepatitis, including chronic active hepatitis, drug fever, and arthralgia.

**Hematologic reactions:** Hemolytic anemia, granulocytopenia, leukopenia, eosinophilia, and megaloblastic anemia. Return of the blood picture to normal has followed cessation of therapy.

**Neurological reactions:** Peripheral neuropathy, headache, dizziness, nystagmus, and drowsiness.

**Miscellaneous reactions:** Transient alopecia. As with other antimicrobial agents, superinfections by resistant organisms may occur. With Macrochantin, however, these are limited to the genitourinary tract because suppression of normal bacterial flora elsewhere in the body does not occur.

Address medical inquiries to: Medical Department, Norwich-Eaton Pharmaceuticals, Norwich, New York 13815

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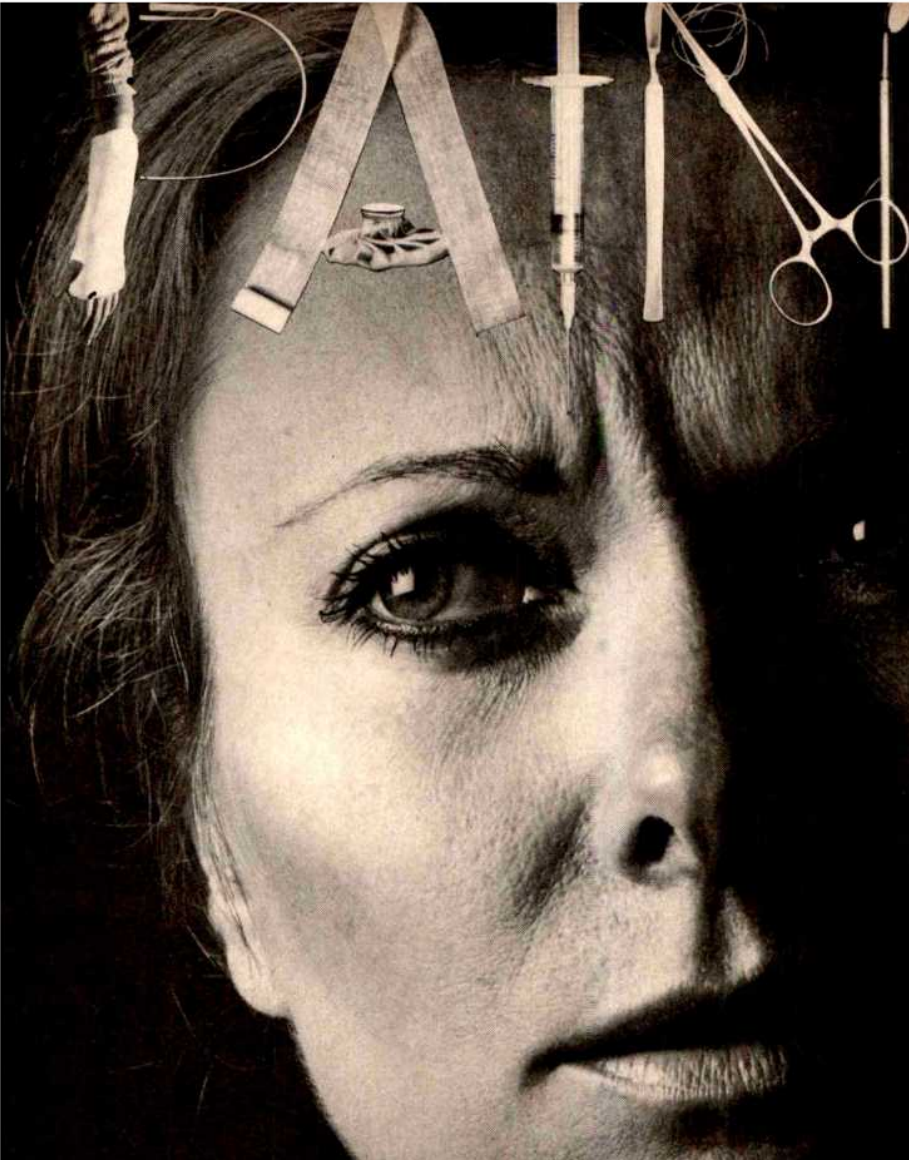


## Contents continued from page 7

|                                                                                                                                                                                                                 |     |
|-----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|-----|
| <b>Amniotic fluid prolactin levels and lecithin/sphingomyelin ratios during the third trimester of human gestation</b>                                                                                          | 435 |
| Christos G. Hatjis, M.D., Chung H. Wu, M.D., and Steven G. Gabbe, M.D.<br><i>Philadelphia, Pennsylvania</i>                                                                                                     |     |
| <b>Fetal metabolic response to endogenous insulin release</b>                                                                                                                                                   | 441 |
| Anthony F. Philipps, Joseph W. Dubin, and John R. Raye<br><i>Farmington, Connecticut</i>                                                                                                                        |     |
| <b>Significance of the sinusoidal fetal heart rate pattern</b>                                                                                                                                                  | 446 |
| Timothy R. B. Johnson, Jr., M.D., Alan A. Compton, M.D., Jacob Rotmensch, M.D.,<br>Bruce A. Work, Jr., M.D., and John W. C. Johnson, M.D.<br><i>Eloise and Ann Arbor, Michigan, and Baltimore, Maryland</i>     |     |
| <b>Corticosteroid suppression of estrogen-induced uterine blood flow in nonpregnant sheep</b>                                                                                                                   | 454 |
| Alan G. Monheit, M.D., and Robert Resnik, M.D.<br><i>La Jolla, California</i>                                                                                                                                   |     |
| <b>Ontogenesis of beta-adrenergic receptors in the ovine placenta</b>                                                                                                                                           | 459 |
| James F. Padbury, M.D., Calvin J. Hobel, M.D., Emmanuel S. Diakomanolis, M.D.,<br>Robert W. Lam, and Delbert A. Fisher, M.D.<br><i>Torrance, California</i>                                                     |     |
| <b>Glucocorticoids, hyperinsulinemia, and fetal lung maturation</b>                                                                                                                                             | 465 |
| J. C. Beck, Ph.D., J. W. C. Johnson, M.D., W. Mitzner, Ph.D., P. A. Lee, M.D.,<br>W. T. London, D.V.M., and D. L. Sly, D.V.M.<br><i>Baltimore and Bethesda, Maryland</i>                                        |     |
| <b>Control of mineral homeostasis during lactation: Interrelationships of 25-hydroxyvitamin D, 24,25-dihydroxyvitamin D, 1,25-dihydroxyvitamin D, parathyroid hormone, calcitonin, prolactin, and estradiol</b> | 471 |
| Laura Hillman, M.D., Sugunamba Sateesha, M.D., Mark Haussler, M.D.,<br>Walter Wiest, Ph.D., Eduardo Slatopolsky, M.D., and John Haddad, M.D.<br><i>St. Louis, Missouri, and Tucson, Arizona</i>                 |     |
| <b>Desoxycorticosterone in normal pregnancy. III. Evidence of a fetal source of desoxycorticosterone</b>                                                                                                        | 477 |
| W. E. Nolten, L. Hughey Holt, and P. A. Rueckert<br><i>Madison, Wisconsin</i>                                                                                                                                   |     |
| <b>An increase in catecholamines and metabolites in the amniotic fluid compartment from middle to late gestation</b>                                                                                            | 483 |
| Walter A. Divers, Jr., M.D., Mahlon M. Wilkes, Ph.D., Ari Babaknia, M.D., and<br>Samuel S. C. Yen, M.D.<br><i>La Jolla, California</i>                                                                          |     |

(Contents continued on page 11)





...may be only  
half the problem.

Apprehension and reaction to pain make up the other very important half. And Synalgos-DC is the analgesic that treats both halves of the pain problem: Promethazine helps relieve apprehension and reaction to pain, while the analgesic components help relieve moderate to moderately severe pain.\*

Synalgos-DC contains dihydrocodeine, a semisynthetic narcotic analgesic related to codeine, with multiple actions qualitatively similar to codeine.

III status permits telephone prescriptions and authorized refills up to 5 times in 6 months (unless restricted by state law). Dual-action Synalgos-DC takes pain off the mind and the mind off pain.

To treat both halves of the problem...

# Synalgos®-DC

Capsules



Drocode (dihydrocodeine) bitartrate 16 mg (Warning: May be habit-forming), promethazine hydrochloride 6.25 mg, aspirin 194.4 mg, phenacetin 162 mg, caffeine 30 mg

**\*INDICATIONS:** Based on a review of this drug by the National Academy of Sciences—National Research Council and/or other information, FDA has classified the indications as follows:

"Possibly" effective for the relief of moderate to moderately severe pain in those situations where the physician wishes to add a mild sedative effect.

Final classification of the less-than-effective indications requires further investigation.

**CONTRAINDICATIONS:** Hypersensitivity to dihydrocodeine, promethazine, aspirin, phenacetin.

**WARNINGS:** Salicylates should be used with extreme caution in the presence of peptic ulcer or coagulation abnormalities.

**Drug Dependence:** Dihydrocodeine can produce drug dependence of the codeine type and therefore has the potential of being abused. Psychic dependence, physical dependence, and tolerance may develop upon repeated administration of dihydro-

codeine, and it should be prescribed and administered with the same degree of caution appropriate to the use of other oral narcotic-containing medications.

Like other narcotic-containing medications, dihydrocodeine is subject to the provisions of the Federal Controlled Substances Act.

**Usage in Ambulatory Patients:** Dihydrocodeine and promethazine may impair the mental and/or physical abilities required for the performance of potentially hazardous tasks such as driving a car or operating machinery. The patient using Synalgos-DC should be cautioned accordingly.

**Interactions with Other Central Nervous System Depressants:** Patients receiving other narcotic analgesics, general anesthetics, other phenothiazines, tranquilizers, sedative-hypnotics, or other CNS depressants (including alcohol) concomitantly with Synalgos-DC may exhibit an additive CNS depression. When such combined therapy is contemplated, the dose of one or both agents should be reduced.

Patients who have demonstrated a hypersensitivity reaction (eg, blood dyscrasia, jaundice) with a

phenothiazine should not be re-exposed to any phenothiazine, including Synalgos-DC, unless in the judgment of the physician the potential benefits of the treatment outweigh the possible hazards.

**Usage in Pregnancy:** Reproduction studies have not been performed in animals. There is no adequate information on whether this drug may affect fertility in human males and females or has a teratogenic potential or other adverse effect on the fetus.

**Usage in Children:** Since there is no experience in children who have received this drug, safety and efficacy in children have not been established.

**PRECAUTIONS:** Phenacetin has been reported to damage kidneys when taken in large amounts for a long time.

Promethazine should be administered cautiously to patients with cardiovascular or liver disease. Synalgos-DC should be given with caution to certain patients such as the elderly or debilitated, and those with hypothyroidism, Addison's disease, and prostatic hypertrophy and urethral stricture.

**ADVERSE REACTIONS:** The most frequently observed

reactions include light-headedness, dizziness, drowsiness, sedation, nausea, vomiting, constipation, pruritus, skin reactions, and rarely, hypotension.

**DOSAGE AND ADMINISTRATION:** Dosage should be adjusted according to the severity of the pain and the response of the patient. Synalgos-DC is given orally. The usual adult dose is two capsules every 4 hours as needed for pain.

**DRUG INTERACTIONS:** The CNS depressant effects of Synalgos-DC may be additive with that of other CNS depressants.

See Warnings.

Aspirin may enhance the effects of anticoagulants and inhibit the uricosuric effects of uricosuric agents.

Consult complete product information before prescribing.

**IVES LABORATORIES INC.**  
685 Third Avenue, New York, NY 10017  
Dedicated to improving the quality of life, through Medicine®





## Contents continued from page 9

---

**Prolactin-stimulated ovarian androgen metabolism** 487

Mary Lake Polan, M.D., Ph.D., and Harold R. Behrman, Ph.D.

*New Haven, Connecticut*

**Elevated free cortisol index in pregnancy: Possible regulatory mechanisms** 492

W. E. Nolton and P. A. Rueckert

*Madison, Wisconsin*

*Information for authors on page 21*

*Index to advertisers on page 48*

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and breakthrough bleeding<sup>†</sup>**

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serious as well as minor side effects have been reported with the use of oral  
contraceptives. These include thromboembolic disease. The physician should  
remain alert to the earliest manifestations of any symptoms of serious disease.

**Please see Complete Prescribing Information,  
a brief summary of which appears on the next page.**

ORTHO



therefore should not be used as the basis for prescribing the product. This summary was prepared by deleting from the complete prescribing information certain text, tables, and references. The physician should be thoroughly familiar with the complete prescribing information and patient information before prescribing the product.

**INDICATION: CONTRACEPTION.** The pregnancy rate in women using conventional combination oral contraceptives (containing 35 mcg or more of ethinyl estradiol or 50 mcg or more of mestranol) is generally reported as less than one pregnancy per 100 woman-years of use. Slightly higher rates (somewhat more than one pregnancy per 100 woman-years of use) are reported for some combination products containing 35 mcg or less of ethinyl estradiol, and rates on the order of three pregnancies per 100 woman-years are reported for the progestogen-only oral contraceptives. Table 1 gives ranges of pregnancy rates reported in the literature for other means of contraception. The efficacy of these means of contraception (except the IUD) depends upon the degree of adherence to the method.

**Table 1. Pregnancy Rates per 100 Woman-Years.** IUD, less than 1-6; Diaphragm with spermicidal product (creams or jellies), 2-20; Condom, 3-36; Aerosol foams, 2-29; Jellies and creams, 4-36; Periodic abstinence (rhythm) all types, less than 1-47; 1. Calendar method, 14-47; 2. Temperature method, 1-20; 3. Temperature method—intercourse only in postovulatory phase, less than 1-7; 4. Mucus method, 1-25; No contraception, 60-80. **DOSE-RELATED RISK OF THROMBOEMBOLISM FROM ORAL CONTRACEPTIVES:** Two studies have shown a positive association between the dose of estrogens in oral contraceptives and the risk of thromboembolism. For this reason, it is prudent and in keeping with good principles of therapeutics to minimize exposure to estrogen. The oral contraceptive product prescribed for any given patient should be that product which contains the least amount of estrogen that is compatible with an acceptable pregnancy rate and patient acceptance. It is recommended that new acceptors of oral contraceptives be started on preparations containing 50 mcg or less of estrogen. **CONTRAINDICATIONS:** Oral contraceptives should not be used in women with any of the following conditions: 1. Thrombophlebitis or thromboembolic disorders. 2. A past history of deep vein thrombophlebitis or thromboembolic disorders. 3. Cerebral vascular or coronary artery disease. 4. Known or suspected carcinoma of the breast. 5. Known or suspected estrogen-dependent neoplasia. 6. Undiagnosed abnormal genital bleeding. 7. Known or suspected pregnancy (see WARNINGS, 5-8). 8. Benign or malignant liver tumor which developed during the use of oral contraceptives or other estrogen-containing products.

#### WARNINGS

**Cigarette smoking increases the risk of serious cardiovascular side effects from oral contraceptive use. This risk increases with age and with heavy smoking (15 or more cigarettes per day) and is quite marked in women over 35 years of age. Women who use oral contraceptives should be strongly advised not to smoke. The use of oral contraceptives is associated with increased risk of several serious conditions including thromboembolism, stroke, myocardial infarction, hepatic adenoma, gallbladder disease, hypertension. Practitioners prescribing oral contraceptives should be familiar with the following information relating to these risks.**

**1. THROMBOEMBOLIC DISORDERS AND OTHER VASCULAR PROBLEMS.** An increased risk of thromboembolic and thrombotic disease associated with the use of oral contraceptives is well established. Four principal studies in Great Britain and three in the United States have demonstrated an increased risk of fatal and nonfatal venous thromboembolism and stroke, both hemorrhagic and thrombotic. These studies estimate that users of oral contraceptives are 4 to 11 times more likely than nonusers to develop these diseases without evident cause. Overall excess mortality due to pulmonary embolism or stroke is on the order of 1.0 to 3.5 deaths annually per 100,000 users and increases with age. **CEREBROVASCULAR DISORDERS:** In a collaborative American study of cerebrovascular disorders in women with and without predisposing causes, it was estimated that the risk of hemorrhagic stroke was 2.0 times greater in users than in nonusers and the risk of thrombotic stroke was 4.0 to 9.5 times greater in users than in nonusers.

**MYOCARDIAL INFARCTION:** An increased risk of myocardial infarction associated with the use of oral contraceptives has been reported confirming a previously suspected association. These studies, conducted in the United Kingdom, found, as expected, that the greater the number of underlying risk factors for coronary artery disease (cigarette smoking, hypertension, hypercholesterolemia, obesity, diabetes, history of pre-eclampsia toxemia), the higher the risk of developing myocardial infarction, regardless of whether the patient was an oral contraceptive user or not. Oral contraceptives, however, were found to be a clear additional risk factor. The annual excess case rate (increased risk) of myocardial infarction (fatal and nonfatal) in oral contraceptive users was estimated to be approximately 7 cases per 100,000 women users in the 30-39 age group and 67 cases per 100,000 women users in the 40-44 age group. In terms of relative risk, it has been estimated that oral contraceptive users who do not smoke (smoking is considered a major predisposing condition to myocardial infarction) are about twice as likely to have a fatal myocardial infarction as nonusers who do not smoke. Oral contraceptive users who are also smokers have about a 5-fold increased risk of fatal infarction compared to users who do not smoke, but about a 10- to 12-fold increased risk compared to nonusers who do not smoke. Furthermore, the amount of smoking is also an important factor. In determining the importance of these relative risks, however, the baseline rates for various age groups must be given serious consideration. The importance of other predisposing conditions mentioned above in determining relative and absolute risks has not yet been quantified. It is quite likely that the same synergistic action exists, but perhaps to a lesser extent. **Risk of Death:** In an analysis of data derived from several national adverse reaction reporting systems, British investigators concluded that the risk of thromboembolism including coronary thrombosis is directly related to the dose of estrogen used in oral contraceptives. Preparations containing 100 mcg or more of estrogen were associated with a higher risk of thromboembolism than those containing 50-80 mcg of estrogen. Their analysis did suggest, however, that the quantity of estrogen may not be the sole factor involved. This finding has been confirmed in the United States. Careful epidemiological studies to determine the degree of thromboembolic risk associated with progestogen-only oral contraceptives have not been performed. Cases of thromboembolic disease have been reported in women using these products, and they should not be presumed to be free of excess risk. The risk of thromboembolic and thrombotic disorders, in both users and nonusers of oral contraceptives, increases with age. Oral contraceptives are, however, an independent risk factor for these events. **ESTIMATE OF EXCESS MORTALITY FROM CIRCULATORY DISEASES:** A large prospective study carried out in the United Kingdom estimated the mortality rate per 100,000 women per year from diseases of the circulatory system for users and nonusers of oral contraceptives according to age, smoking habits, and duration of use. The overall excess death rate annually from circulatory diseases for oral contraceptive users was estimated to be 20 per 100,000 (ages 15-34—5/100,000; ages 35-44—33/100,000; ages 45-49—140/100,000), the risk being concentrated in older women, in those with a long duration of use, and in cigarette smokers. This was not possible, however, to examine the interrelationships of age, smoking, and duration of use, nor to compare the effects of continuous versus intermittent use. Although the study showed a 10-fold increase in death due to circulatory diseases in users for five or more years, all of these deaths occurred in women 35 or older. Until larger numbers of women under 35 with continuous use for five or more years are available, it is not possible to assess the magnitude of the relative risk for this younger age group. Another study published at the same time confirms a previously reported increase of mortality in pill users from cardiovascular disease. The available data from a variety of sources have been analyzed to estimate the risk of death associated with various methods of contraception. The estimates of risk of death for each method include the combined risk of the contraceptive method (e.g., thromboembolic and thrombotic disease in the case of oral contraceptives) plus the risk attributable to pregnancy or abortion in the event of method failure. This latter risk varies with the effectiveness of the contraceptive method. The findings of this analysis are shown in Figure 1 below. The study concluded that the mortality associated with all methods of birth control is low and below that associated with childbirth. With the exception of oral contraceptives in women over 40 who smoke, (The rates given for pill only/smokers for each age group are for smokers as a class. For "heavy" smokers [more than 15 cigarettes a day], the rates given would be about double; for "light" smokers [less than 15 cigarettes a day], about 50 percent.) The mortality associated with oral contraceptive use in nonsmokers over 40 is higher than with any other method of contraception in that age group. The lowest mortality is associated with the condom or diaphragm backed up by early abortion. The risk of thromboembolic and thrombotic disease associated with oral contraceptives increases with age after approximately age 30 and, for myocardial infarction, is further increased by hypertension, hypercholesterolemia, obesity, diabetes, or history of pre-eclampsia toxemia and especially by cigarette smoking. The risk of myocardial infarction in oral contraceptive users is substantially increased in women age 40 and over, especially those with any risk factors. Based on the data currently available, the following chart gives a gross estimate of the risk of death from circulatory disorders associated with the use of oral contraceptives.

#### SMOKING HABITS AND OTHER PREDISPOSING CONDITIONS—RISK ASSOCIATED WITH USE OF ORAL CONTRACEPTIVES

| Age                                        | Below 30 | 30-39 | 40+ |
|--------------------------------------------|----------|-------|-----|
| Heavy smokers                              | C        | B     | A   |
| Light smokers                              | D        | C     | B   |
| Nonsmokers (no predisposing conditions)    | C        | D     | C   |
| Nonsmokers (other predisposing conditions) | C        | B     | A   |

The physician and the patient should be alert to the earliest manifestations of thromboembolic and thrombotic disorders (e.g., thrombophlebitis, pulmonary embolism, cerebrovascular insufficiency, coronary occlusion, retinal thrombosis, and mesenteric thrombosis). Should any of these occur or be suspected, the drug should be discontinued immediately. A four- to six-fold increased risk of postsurgery thromboembolic complications has been reported in oral contraceptive users. If feasible, oral contraceptives should be discontinued at least four weeks before surgery of a type associated with an increased risk of thromboembolism or prolonged immobilization. **2. OCULAR LESIONS:** There have been reports of neuro-ocular lesions such as optic neuritis or retinal thrombosis associated with the use of oral contraceptives. Discontinue oral contraceptive medication if there is unexplained, sudden or gradual, partial or complete loss of vision; onset of proptosis or diplopia; papilledema; or retinal vascular lesions and institute appropriate diagnostic and therapeutic measures. **3. CARCINOMA:** Long-term continuous administration of either natural or synthetic estrogen in certain animal species increases the frequency of carcinoma of the breast, cervix, vagina, and liver. Certain synthetic progestogens, none currently contained in oral contraceptives, have been noted to increase the incidence of mammary nodules, benign and malignant, in dogs. In humans, three case control studies have reported an increased risk of endometrial carcinoma associated with the prolonged use of exogenous estrogen in postmenopausal women. One publication reported on the first 21 cases submitted by physicians to a registry of cases of adenocarcinoma of the endometrium in women under 40 on oral contraceptives. Of the cases found in women without predisposing risk factors for adenocarcinoma of the endometrium (e.g., irregular bleeding at the time oral contraceptives were first given, polycystic ovaries), nearly all occurred in women who had used a sequential oral contraceptive. These products are no longer marketed. No evidence has been reported suggesting an increased risk of endometrial cancer in users of conventional combination or progestogen-only oral contraceptives. Several studies have found no increases in breast cancer in women taking oral contraceptives or estrogens. One study, however, while also noting no overall increased risk of breast cancer in women treated with oral contraceptives, found an excess risk in the subgroups of oral contraceptive users with documented benign breast disease. A reduced occurrence of benign breast tumors in users of oral contraceptives has been well-documented. In summary, there is at present no confirmed evidence from human studies of an increased risk of cancer associated with oral contraceptives. Close clinical surveillance of all women taking oral contraceptives is, nevertheless, essential. In all cases of undiagnosed persistent or recurrent abnormal vaginal bleeding, appropriate diagnostic measures should be taken to rule out malignancy. Women with a strong family history of breast cancer or who have breast nodules, fibrocystic disease, or abnormal mammograms should be monitored with particular care if they elect to use oral contraceptives instead of other methods of contraception. \*Trademark

|                                        | 15-19 | 20-24 | 25-29 | 30-34 | 35-39 | 40-44 |
|----------------------------------------|-------|-------|-------|-------|-------|-------|
| No method                              | 5.5   | 5.2   | 7.1   | 14.0  | 19.3  | 21.9  |
| Abortion only                          | 2.3   | 2.5   | 2.5   | 5.2   | 9.8   | 6.6   |
| Pill only-nonsmokers                   | 1.3   | 1.4   | 1.4   | 2.2   | 4.5   | 3.1   |
| Pill only-smokers                      | 1.5   | 1.6   | 1.6   | 10.8  | 13.4  | 58.9  |
| IUDs only                              | 1.1   | 1.2   | 1.2   | 1.4   | 1.6   | 1.4   |
| Traditional contraception only         | 1.1   | 1.4   | 1.9   | 3.7   | 4.7   | 4.0   |
| Traditional contraception and abortion | 0.3   | 0.4   | 0.4   | 0.8   | 1.4   | 0.8   |

**4. HEPATIC TUMORS:** Benign hepatic adenomas have been found to be associated with the use of oral contraceptives. One study showed that oral contraceptive formulations with high hormonal potency were associated with a higher risk than lower potency formulations and use of oral contraceptives with high hormonal potency and age over 30 years may further increase the woman's risk of hepatocellular adenoma. Although benign, hepatic adenomas may rupture and may cause death through intra-abdominal hemorrhage. This has been reported in short-term as well as long-term users of oral contraceptives. Two studies relate risk with duration of use of the contraceptive, the risk being much greater after four or more years of oral contraceptive use. While hepatic adenoma is a rare lesion, it should be considered in women presenting abdominal pain and tenderness, abdominal mass or shock. A few cases of hepatocellular carcinoma have been reported in women taking oral contraceptives. The relationship of these drugs to this type of malignancy is not known at this time. **5. USE IN OR IMMEDIATELY PRECEDING PREGNANCY, BIRTH DEFECTS IN OFFSPRING, AND MALIGNANCY IN FEMALE OFFSPRING:** The use of female sex hormones—both estrogen and progestational agents—during early pregnancy may seriously damage the offspring. It has been shown that females exposed in utero to diethylstilbestrol, a nonsteroidal estrogen, have an increased risk of developing in later life a form of vaginal or cervical cancer that is ordinarily extremely rare. This risk has been estimated to be on the order of 1 to 4 in 1000 exposures. Although there is no evidence at the present time that oral contraceptives further enhance the risk of developing this type of malignancy, such patients should be monitored with particular care if they elect to use oral contraceptives instead of other methods of contraception. Furthermore, a high percentage of such exposed women (from 30 to 90%) have been found to have epithelial changes of the vagina and cervix. Although these changes are histologically benign, it is not known whether this condition is a precursor of vaginal malignancy. Male children so exposed may develop abnormalities of the urogenital tract. Although similar data are not available with the use of other estrogens, it cannot be presumed that they would not induce similar changes. An increased risk of congenital anomalies, including heart defects and limb defects, has been reported with the use of sex hormones, including oral contraceptives, in pregnancy. One case control study has estimated a 4.7-fold increase in risk of limb-reduction defects in infants exposed in utero to sex hormones (oral contraceptives, hormonal withdrawal tests for pregnancy or attempted treatment for threatened abortion). Some of these exposures were very short and involved only a few days of treatment. The data suggest that the risk of limb-reduction defects in exposed fetuses is somewhat less than one in 1000 live births. In the past, female sex hormones have been used during pregnancy in an attempt to treat threatened or habitual abortion. There is considerable evidence that estrogens are ineffective for these indications, and there is no evidence from well-controlled studies that progestogens are effective for these uses. There is some evidence that triploidy and possibly other types of polyploidy are increased among abortuses from women who become pregnant soon after ceasing oral contraceptives. Embryos with these anomalies are virtually always aborted spontaneously. Whether there is an overall increase in spontaneous abortion of pregnancies conceived soon after stopping oral contraceptives is unknown. It is recommended that for any patient who has missed two consecutive periods, pregnancy should be ruled out before continuing the contraceptive regimen. If the patient has not adhered to the prescribed schedule, the possibility of pregnancy should be considered at the time of the first missed period (or after 45 days from the last menstrual period if the progestogen-only oral contraceptives are used), and further use of oral contraceptives should be withheld until pregnancy has been ruled out. If pregnancy is confirmed, the patient should be apprised of the potential risks to the fetus and the advisability of continuation of the pregnancy should be discussed in the light of these risks. It is also recommended that women who discontinue oral contraceptives with the intent of becoming pregnant use an alternate form of contraception for a period of time before attempting to conceive. Many clinicians recommend three months although no precise information is available on which to base this recommendation. The administration of progestogen-only or progestogen-estrogen combinations to induce withdrawal bleeding should not be used as a test of pregnancy. **6. GALLBLADDER DISEASE:** Studies report an increased risk of surgically confirmed gallbladder disease in users of oral contraceptives and estrogens. In one study, an increased risk appeared after two years of use and doubled after four or five years of use. In one of the other studies, an increased risk was apparent between six and twelve months of use. **7. CARBOHYDRATE AND LIPID METABOLIC EFFECTS:** A decrease in glucose tolerance has been observed in a significant percentage of patients on oral contraceptives. For this reason, prediabetic and diabetic patients should be carefully observed while receiving oral contraceptives. An increase in triglycerides and total phospholipids has been observed in patients receiving oral contraceptives. The clinical significance of this finding remains to be defined. **8. ELEVATED BLOOD PRESSURE:** An increase in blood pressure has been reported in patients receiving oral contraceptives. In some women hypertension may occur within a few months of beginning oral contraceptive use. In the first year of use, the prevalence of women with hypertension is low in users and may be no higher than that of a comparable group of nonusers. The prevalence in users increases, however, with longer exposure, and in the fifth year of use is two and a half to three times the reported prevalence in the first year. Age is also strongly correlated with the development of hypertension in oral contraceptive users. Women who previously have had hypertension during pregnancy may be more likely to develop elevation of blood pressure when given oral contraceptives. Hypertension that develops as a result of taking oral contraceptives usually returns to normal after discontinuing the drug. **9. HEADACHE:** The onset or exacerbation of migraine or development of headache of a new pattern which is recurrent, persistent, or severe, requires discontinuation of oral contraceptives and evaluation of the cause. **10. BLEEDING IRREGULARITIES:** Breakthrough bleeding, spotting, and amenorrhea are frequent reasons for patients discontinuing oral contraceptives. In breakthrough bleeding, as in all cases of irregular bleeding from the vagina, nonfunctional causes should be borne in mind. In undiagnosed persistent or recurrent abnormal bleeding from the vagina, adequate diagnostic measures are indicated to rule out pregnancy or malignancy. If pathology has been excluded, time or a change to another formulation may solve the problem. Changing to an oral contraceptive with a higher estrogen content, while potentially useful in minimizing menstrual irregularity, should be done only if necessary since this may increase the risk of thromboembolic disease. Women with a past history of oligomenorrhea or secondary amenorrhea or young women without regular cycles may have a tendency to remain anovulatory or to become amenorrheic after discontinuation of oral contraceptives. Women with these preexisting problems should be advised of this possibility and encouraged to use other contraceptive methods. Postuse anovulation, possibly prolonged, may also occur in women without previous irregularities. **11. ECTOPIC PREGNANCY:** Ectopic as well as intrauterine pregnancy may occur in contraceptive failures. **12. BREAST FEEDING:** Oral contraceptives given in the postpartum period may interfere with lactation. There may be a decrease in the quantity and quality of the breast milk. Furthermore, a small fraction of the hormonal agents in oral contraceptives has been identified in the milk of mothers receiving these drugs. The effects, if any, on the breast-fed child have not been determined. If feasible, the use of oral contraceptives should be deferred until the infant has been weaned. **PRECAUTIONS: General:** 1. A complete medical and family history should be taken prior to the initiation of oral contraceptives. The pretreatment and periodic physical examinations should include special reference to blood pressure, breasts, abdomen and pelvic organs, including Papanicolaou smear and relevant laboratory tests. As a general rule, oral contraceptives should not be prescribed for longer than one year without another physical examination being performed. 2. Under the influence of estrogen-progestogen preparations, preexisting uterine leiomyomata may increase in size. 3. Patients with a history of psychic depression should be carefully observed and the drug discontinued if depression recurs to a serious degree. Patients becoming significantly depressed while taking oral contraceptives should stop the medication and use an alternate method of contraception in an attempt to determine whether the symptom is drug-related. 4. Oral contraceptives may cause some degree of fluid retention. They should be prescribed with caution, and only with careful monitoring, in patients with conditions which might be aggravated by fluid retention, such as convulsive disorders, migraine syndrome, asthma, or cardiac or renal insufficiency. 5. Patients with a past history of jaundice during pregnancy have an increased risk of recurrence of jaundice while receiving oral contraceptive therapy. If jaundice develops in any patient receiving such drugs, the medication should be discontinued. 6. Steroid hormones may be poorly metabolized in patients with impaired liver function and should be administered with caution in such patients. 7. Oral contraceptive users may have disturbances in normal tryptophan metabolism which may result in a relative pyridoxine deficiency. The clinical significance of this is yet to be determined. 8. Serum folate levels may be depressed by oral contraceptive therapy. Since the pregnant woman is predisposed to the development of folate deficiency and the incidence of folate deficiency increases with increasing gestation, it is possible that if a woman becomes pregnant shortly after stopping oral contraceptives, she may have a greater chance of developing folate deficiency and complications attributed to this deficiency. 9. The pathologist should be advised of oral contraceptive therapy when relevant specimens are submitted. 10. Certain endocrine and liver function tests and blood components may be affected by estrogen-containing oral contraceptives: a. Increased sulfobromophthalen retention. b. Increased prothrombin and factors VII, VIII, IX, and X; decreased antithrombin. c. Increased norepinephrine-induced platelet aggregability. d. Increased thyroid-binding globulin (TBG) leading to increased circulating total thyroid hormone, as measured by protein-bound iodine (PBI), T4 by column, or T4 by radioimmunoassay. Free T3 resin uptake is decreased, reflecting the elevated TBG. Free T4 concentration is unaltered. d. Decreased pregnandiol excretion. e. Reduced response to metoprolol test. **INFORMATION FOR THE PATIENT: [See Patient Package Insert]. DRUG INTERACTIONS:** Reduced efficacy and increased incidence of breakthrough bleeding have been associated with concomitant use of rifampin. A similar association has been suggested with barbiturates, phenylbutazone, phenyltolonamide, ampicillin, and tetracycline. **CARCINOGENESIS, PREGNANCY, NURSING MOTHERS:** See CONTRAINDICATIONS and WARNINGS. **ADVERSE REACTIONS:** An increased risk of the following serious adverse reactions has been associated with the use of oral contraceptives (see WARNINGS): Thrombophlebitis. Pulmonary embolism. Coronary thrombosis. Cerebral thrombosis. Cerebral hemorrhage. Hypertension. Gallbladder disease. Liver tumors. Congenital anomalies. There is evidence of an association between the following conditions and the use of oral contraceptives, although additional confirmatory studies are needed: Mesenteric thrombosis. Neuro-ocular lesions, e.g., retinal thrombosis and optic neuritis. The following adverse reactions have been reported in patients receiving oral contraceptives and are believed to be drug-related: Nausea, usually the most common adverse reaction. Vomiting, occurs in approximately 10% or less of patients during the first cycle. Other reactions, as a general rule, are seen much less frequently or only occasionally. Gastrointestinal symptoms (such as abdominal cramps and bloating). Breakthrough bleeding. Spotting. Change in menstrual flow. Dysmenorrhea. Amenorrhea during and after treatment. Temporary infertility after discontinuance of treatment. Edema. Chloasma or melasma which may persist. Breast changes: tenderness, enlargement, and secretion. Change in weight (increase or decrease). Change in cervical erosion and cervical secretion. Possible diminution in lactation when given immediately postpartum. Cholestatic jaundice. Migraine: increase in size of uterine leiomyomata. Rash (allergic). Mental depression. Reduced tolerance to carbohydrates. Vaginal candidiasis. Change in corneal curvature (steepening). Intolerance to contact lenses. The following adverse reactions have been reported in users of oral contraceptives, and the association has been neither confirmed nor refuted: Premenstrual-like syndrome. Cataracts. Changes in libido. Chorea. Changes in appetite. Ovarian-like syndrome. Headache. Nervousness. Dizziness. Hirsutism. Loss of scalp hair. Erythema multiforme. Erythema nodosum. Hemorrhagic eruption. Vaginitis. Porphyria. Impaired renal function. **ACUTE OVERDOSE:** Serious ill effects have not been reported following acute ingestion of large doses of oral contraceptives by young children. Overdose may cause nausea, and withdrawal bleeding may occur in females.





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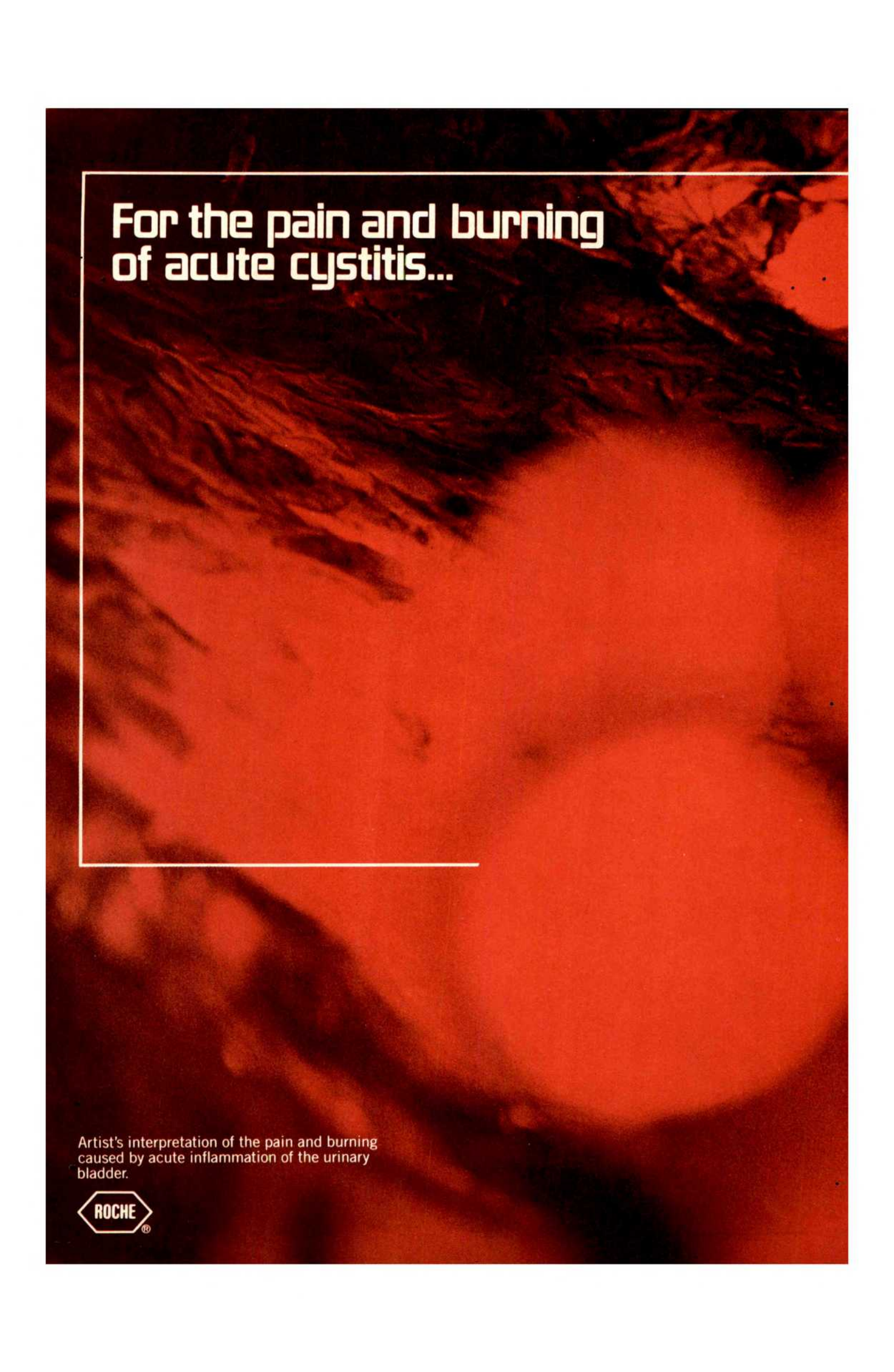
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**For the pain and burning  
of acute cystitis...**

Artist's interpretation of the pain and burning  
caused by acute inflammation of the urinary  
bladder.





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**In nearly 9 out of 10 patients,  
Azo Gantanol reduced the  
pain and/or burning of acute  
cystitis within 24 hours.\***

A controlled, multicenter study assessed the efficacy of Azo Gantanol in relieving the pain and/or burning associated with acute urinary tract infection. Patients included in the study had at least 100,000 colonies per ml of a sulfonamide-sensitive organism, usually *E. coli*. In 87% of patients (303 of 349) with initial symptoms rated "moderate to severe," therapy with Azo Gantanol resulted in improvement within 24 hours.†

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† Data on file, Hoffmann-La Roche Inc., Nutley, New Jersey 07110

**4 tablets initially followed by 2 tablets B.I.D.**

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Each tablet contains 0.5 Gm sulfamethoxazole and 100 mg phenazopyridine HCl.

**for  
the pain—**

**for  
the pathogens**

\*Azo Gantanol recommended for relief of pain, followed by Gantanol (sulfamethoxazole/Roche) for remaining course of therapy.

Please see brief summary of complete product information on following page.



# Azo Gantanol®

Each tablet contains 0.5 Gm sulfamethoxazole and 100 mg phenazopyridine HCl.

Before prescribing, please consult complete product information, a summary of which follows:

**INDICATIONS:** In adults, urinary tract infections complicated by pain (primarily pyelonephritis, pyelitis and cystitis) due to susceptible organisms (usually *E. coli*, *Klebsiella-Aerobacter*, *Staphylococcus aureus*, *Proteus mirabilis*, and, less frequently, *Proteus vulgaris*) in the absence of obstructive uropathy or foreign bodies.

**Note:** Carefully coordinate *in vitro* sulfonamide sensitivity tests with bacteriologic and clinical response; add aminobenzoic acid to follow-up culture media. The increasing frequency of resistant organisms limits the usefulness of antibacterials including sulfonamides. Measure sulfonamide blood levels as variations may occur; 20 mg/100 ml should be maximum total level.

**CONTRAINDICATIONS:** Children below age 12; sulfonamide hypersensitivity; pregnancy at term and during nursing period; because Azo Gantanol contains phenazopyridine hydrochloride it is contraindicated in glomerulonephritis, severe hepatitis, uremia, and pyelonephritis of pregnancy with G.I. disturbances.

**WARNINGS:** Safety during pregnancy not established. Deaths from hypersensitivity reactions, agranulocytosis, aplastic anemia and other blood dyscrasias have been reported and early clinical signs (sore throat, fever, pallor, purpura or jaundice) may indicate serious blood disorders. Frequent CBC and urinalysis with microscopic examination are recommended during sulfonamide therapy.

**PRECAUTIONS:** Use cautiously in patients with impaired renal or hepatic function, severe allergy, bronchial asthma; in glucose-6-phosphate dehydrogenase-deficient individuals in whom dose-related hemolysis may occur. Maintain adequate fluid intake to prevent crystalluria and stone formation.

**ADVERSE REACTIONS:** *Blood dyscrasias* (agranulocytosis, aplastic anemia, thrombocytopenia, leukopenia, hemolytic anemia, purpura, hypoprothrombinemia and methemoglobinemia); *allergic reactions* (erythema multiforme, Stevens-Johnson syndrome, skin eruptions, epidermal necrolysis, urticaria, serum sickness, pruritus, exfoliative dermatitis, anaphylactoid reactions, periorbital edema, conjunctival and scleral injection, photosensitization, arthralgia and allergic myocarditis); *G.I. reactions* (nausea, emesis, abdominal pains, hepatitis, diarrhea, anorexia, pancreatitis and stomatitis); *CNS reactions* (headache, peripheral neuritis, mental depression, convulsions, ataxia, hallucinations, tinnitus, vertigo and insomnia); *miscellaneous reactions* (drug fever, chills, toxic nephrosis with oliguria and anuria, periarteritis nodosa and L. E. phenomenon). Due to certain chemical similarities with some goitrogens, diuretics (acetazolamide, thiazides) and oral hypoglycemic agents, sulfonamides have caused rare instances of goiter production, diuresis and hypoglycemia. Cross-sensitivity with these agents may exist.

**DOSAGE:** Azo Gantanol is intended for the acute, painful phase of urinary tract infections. *Usual adult dosage:* 2 Gm (4 tabs) initially, then 1 Gm (2 tabs) B.I.D. for up to 3 days. If pain persists, causes other than infection should be sought. After relief of pain has been obtained, continued treatment with Gantanol (sulfamethoxazole/Roche) may be considered.

**NOTE:** Patients should be told that the orange-red dye (phenazopyridine HCl) will color the urine.

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Tables should be typed on separate sheets of paper, not in the text, with one table to a page. They should be numbered in sequence and each must be referred to at an appropriate point in the text. Captions of the tables should be brief, yet indicate clearly the purpose or content of each table. Rows and columns in the table should precisely define the nature of the data in each. Abbreviations in tables should be used as little as possible and if abbreviations are used they should be explained in a footnote to the table.

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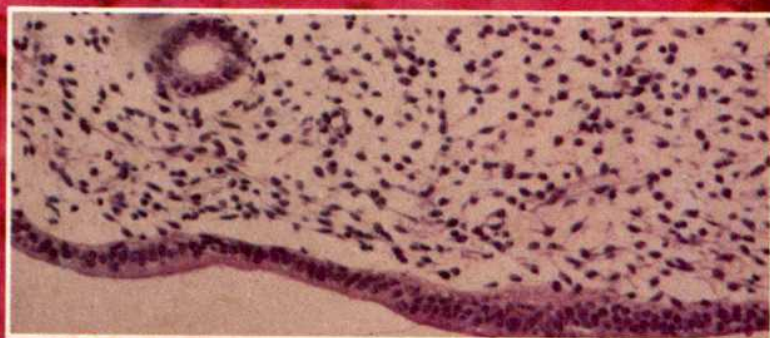
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each tablet contains 0.3 mg norgestrel with 0.03 mg ethinyl estradiol, Wyeth

...with a near-spotless record!

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**Indications and Usage**—LOOVRAL is indicated for the prevention of pregnancy in women who elect to use oral contraceptives (OC's) as a method of contraception.

**Contraindications**—OC's should not be used in women with any of the following conditions: 1. Thrombophlebitis or thromboembolic disorders. 2. A past history of deep-vein thrombophlebitis or thromboembolic disorders. 3. Cerebral-vascular or coronary-artery disease. 4. Known or suspected carcinoma of the breast. 5. Known or suspected estrogen-dependent neoplasia. 6. Undiagnosed abnormal genital bleeding. 7. Known or suspected pregnancy (see Warning No. 5). 8. Benign or malignant liver tumor which developed during use of OC's or other estrogen-containing products.

#### Warnings

**Cigarette smoking increases the risk of serious cardiovascular side effects from oral contraceptive use. This risk increases with age and with heavy smoking (15 or more cigarettes per day) and is quite marked in women over 35 years of age. Women who use oral contraceptives should be strongly advised not to smoke.**

The use of oral contraceptives is associated with increased risk of several serious conditions, including thromboembolism, stroke, myocardial infarction, hepatic adenoma, gallbladder disease, hypertension. Practitioners prescribing oral contraceptives should be familiar with the following information relating to these risks.

1. **Thromboembolic Disorders and Other Vascular Problems**—An increased risk of thromboembolic and thrombotic disease associated with use of OC's is well established. Three principal studies in Great Britain and 3 in the U.S. have demonstrated increased risk of fatal and nonfatal venous thromboembolism and stroke, both hemorrhagic and thrombotic. These studies estimate that users of OC's are 4 to 11 times more likely than nonusers to develop these diseases without evident cause.

**CEREBROVASCULAR DISORDERS**—In a collaborative American study of cerebrovascular disorders in women with and without predisposing causes, it was estimated that the risk of hemorrhagic stroke was 2.0 times greater in users than nonusers and the risk of thrombotic stroke was 4 to 9.5 times greater in users than in nonusers.

**MYOCARDIAL INFARCTION (MI)**—An increased risk of MI associated with use of OC's has been reported, confirming a previously suspected association. These studies, conducted in the UK, found, as expected, that the greater the number of underlying risk factors for coronary artery disease (cigarette smoking, hypertension, hypercholesterolemia, obesity, diabetes, history of pre-eclamptic toxemia) the higher the risk of developing MI, regardless of whether the patient was an OC user or not. OC's, however, were found to be a clear additional risk factor. In terms of relative risk, it has been estimated that OC users who do not smoke (smoking is considered a major predisposing condition to MI) are about twice as likely to have a fatal MI as nonusers who do not smoke. OC users who are also smokers have about a 5-fold increased risk of fatal MI compared to users who do not smoke, but about a 10- to 12-fold increased risk compared to nonusers who do not smoke. Furthermore, amount of smoking is also an important factor. In determining importance of these relative risks, however, baseline rates for various age groups must be given serious consideration. Importance of other predisposing conditions mentioned above in determining relative and absolute risks has not as yet been quantified; quite likely the same synergistic action exists, but perhaps to a lesser extent.

**RISK OF DOSE**—In an analysis of data derived from several national adverse-reaction reporting systems, British investigators concluded that risk of thromboembolism, including coronary thrombosis, is directly related to dose of estrogen in OC's. Preparations containing 100 mcg or more of estrogen were associated with higher risk of thromboembolism than those containing 50-80 mcg. Their analysis did suggest, however, that quantity of estrogen may not be the sole factor involved. This finding has been confirmed in the U.S.

**ESTIMATE OF EXCESS MORTALITY FROM CIRCULATORY DISEASES**—A large prospective study carried out in the UK estimated the mortality rate per 100,000 women per year from diseases of the circulatory system for users and nonusers of OC's according to age, smoking habits, and duration of use. Overall excess death rate annually from circulatory diseases for OC users was estimated to be 20 per 100,000 (ages 15-34—5,100,000; ages 35-44—33,100,000; ages 45-49—140,100,000), risk being concentrated in older women, in those with long duration of use, and in cigarette smokers. It was not possible, however, to examine interrelationships of age, smoking, and duration of use, nor to compare effects of continuous vs. intermittent use. Although the study showed a 10-fold increase in death due to circulatory diseases in users for 5 or more years, all these deaths occurred in women 35 or older. Until larger numbers of women under 35 with continuous use for 5 or more years are available, it is not possible to assess magnitude of relative risk for this younger group. Available data from a variety of sources have been analyzed to estimate risk of death associated with various methods of contraception. Estimates of risk of death for each method include combined risk of contraceptive method (e.g., thromboembolic and thrombotic disease in the case of OC's) plus risk attributable to pregnancy or abortion in event of method failure. This latter risk varies with effectiveness of method. The study concluded that mortality associated with all methods of birth control is low and below that associated with childbirth, with the exception of OC's in women over 40 who smoke. Lowest mortality is associated with condom or diaphragm backed up by early abortion. Risk of thromboembolic and thrombotic disease associated with OC's increases with age after about 30 and, for MI, is further increased by hypertension, hypercholesterolemia, obesity, diabetes, or history of pre-eclamptic toxemia, and especially cigarette smoking. Physician and patient should be alert to earliest manifestations of thromboembolic and thrombotic disorders (e.g., thrombophlebitis, pulmonary embolism, cerebrovascular insufficiency, coronary occlusion, retinal thrombosis, and mesenteric thrombosis). Should any of these occur or be suspected, the drug should be discontinued immediately.

A 4- to 6-fold increased risk of postsurgery thromboembolic complications has been reported in OC users.

If feasible, OC's should be discontinued at least 4 weeks before surgery of a type associated with increased risk of thromboembolism or prolonged immobilization.

2. **Ocular Lesions**—There have been reports of neuro-ocular lesions such as optic neuritis or retinal thrombosis associated with use of OC's. Discontinue OC's if there is unexplained, sudden or gradual, partial or complete loss of vision; onset of proptosis or diplopia; papilledema; or retinal-vascular lesions, and institute appropriate diagnostic and therapeutic measures.

3. **Carcinoma**—Long-term continuous administration of either natural or synthetic estrogen in certain animal species increases frequency of carcinoma of the breast, cervix, vagina, and liver. Certain synthetic progestogens, none currently contained in OC's, have been noted to increase incidence of mammary nodules, benign

reported an increased risk of endometrial carcinoma associated with prolonged use of exogenous estrogen in postmenopausal women. One publication reported on the first 21 cases submitted by physicians to a registry of cases of adenocarcinoma of the endometrium in women under 40 on OC's. Of cases found in women without predisposing risk factors (e.g., irregular bleeding at the time OC's were first given, polycystic ovaries), nearly all occurred in women who had used a sequential OC. These are no longer marketed. No evidence has been reported suggesting increased risk of endometrial cancer in users of conventional combination or progestogen-only OC's. Several studies have found no increase in breast cancer in women taking OC's or estrogens. One study, however, while also noting no overall increased risk of breast cancer in women on OC's, found an excess risk in subgroups of OC users with documented benign breast disease. Reduced occurrence of benign breast tumors in users of OC's has been well documented. In summary, there is at present no confirmed evidence from human studies of increased risk of cancer associated with OC's. Close clinical surveillance of all women on OC's is, nevertheless, essential. In all cases of undiagnosed persistent or recurrent abnormal vaginal bleeding, appropriate diagnostic measures should be taken to rule out malignancy. Women with a strong family history of breast cancer or with breast nodules, fibrocystic disease, or abnormal mammograms should be monitored with particular care if they elect to use OC's.

4. **Hepatic Tumors**—Benign hepatic adenomas have been found to be associated with use of OC's. One study showed that OC's with high hormonal potency were associated with higher risk than lower potency OC's. Although benign, hepatic adenomas may rupture and may cause death through intra-abdominal hemorrhage. This has been reported in short-term as well as long-term users. Two studies relate risk with duration of use of OC's, the risk being much greater after 4 or more years' use. While hepatic adenoma is rare, it should be considered in women presenting abdominal pain and tenderness, abdominal mass or shock. A few cases of hepatocellular carcinoma have been reported in women on OC's. Relationship of these drugs to this type of malignancy is not known.

5. **Use in or Immediately Preceding Pregnancy, Birth Defects in Offspring, and Malignancy in Female Offspring**—Use of female sex hormones—both estrogenic and progestational agents—during early pregnancy may seriously damage the offspring. It has been shown that females exposed in utero to diethylstilbestrol, a nonsteroidal estrogen, have increased risk of developing in later life a form of vaginal or cervical cancer ordinarily extremely rare. This risk has been estimated to be of the order of 1 in 1,000 exposures or less. Although there is no evidence now that OC's further enhance risk of developing this type of malignancy, such patients should be monitored with particular care if they elect to use OC's. Furthermore, 30 to 90% of such exposed women have been found to have epithelial changes of the vagina and cervix. Although these changes are histologically benign, it is not known whether this condition is a precursor of vaginal malignancy. Male children so exposed may develop abnormalities of the urogenital tract. Although similar data are not available with use of other estrogens, it cannot be presumed they would not induce similar changes. An increased risk of congenital anomalies, including heart defects and limb defects, has been reported with use of sex hormones, including OC's, in pregnancy. One case-control study estimated a 4.7-fold increase in risk of limb-reduction defects in infants exposed in utero to sex hormones (OC's, hormonal withdrawal tests for pregnancy, or attempted treatment for threatened abortion). Some exposures involved only a few days. Data suggest that risk of limb-reduction defects in exposed fetuses is somewhat less than 1 in 1,000 live births. In the past, female sex hormones have been used during pregnancy in an attempt to treat threatened or habitual abortion. There is considerable evidence that estrogens are ineffective for these indications, and there is no evidence from well-controlled studies that progestogens are effective. There is some evidence that triploidy and possibly other types of polyploidy are increased among abortions from women who become pregnant soon after ceasing OC's. Embryos with these anomalies are virtually always aborted spontaneously. Whether there is an overall increase in spontaneous abortion of pregnancies conceived soon after stopping OC's is unknown. It is recommended that, for any patient who has missed 2 consecutive periods, pregnancy should be ruled out before continuing OC's. If the patient has not adhered to the prescribed schedule, the possibility of pregnancy should be considered at time of first missed period, and further use of OC's should be withheld until pregnancy has been ruled out. If pregnancy is confirmed, the patient should be apprised of the potential risks to the fetus, and advisability of continuation of the pregnancy should be discussed. It is also recommended that women who discontinue OC's with intent of becoming pregnant use an alternate form of contraception for a period of time before attempting to conceive. Many clinicians recommend 3 months, although no precise information is available on which to base this. The administration of progestogen-estrogen combinations to induce withdrawal bleeding should not be used as a test of pregnancy.

6. **Gallbladder Disease**—Studies report increased risk of surgically confirmed gallbladder disease in users of OC's and estrogens. In one study, increased risk appeared after 2 years' use and doubled after 4 or 5 years' use. In one of the other studies, increased risk was apparent between 6 and 12 months' use.

7. **Carbohydrate and Lipid Metabolic Effects**—Decrease in glucose tolerance has been observed in a significant percentage of patients on OC's. For this reason, prediabetic and diabetic patients should be carefully observed while on OC's. Increase in triglycerides and total phospholipids has been observed in patients on OC's; clinical significance of this finding remains to be defined.

8. **Elevated Blood Pressure**—Increase in blood pressure has been reported in patients on OC's. In some women, hypertension may occur within a few months of beginning OC's. In the 1st year of use, prevalence of women with hypertension is low in users and may be no higher than that of a comparable group of nonusers. Prevalence in users increases, however, with longer exposure, and in the 5th year of use is 2½ to 3 times the reported prevalence in the 1st year. Age is also strongly correlated with development of hypertension in OC users.

Women who previously have had hypertension during pregnancy may be more likely to develop elevation of blood pressure on OC's. Hypertension that develops as a result of taking OC's usually returns to normal after discontinuing the drug.

9. **Headache**—Onset or exacerbation of migraine or development of headache of a new pattern which is recurrent, persistent, or severe, requires discontinuation of OC's and evaluation of the cause.

10. **Bleeding Irregularities**—Breakthrough bleeding, spotting, and amenorrhea are frequent reasons for patients discontinuing OC's. In breakthrough bleeding, as in all cases of irregular

the vagina, adequate diagnostic measures are indicated to rule out pregnancy or malignancy. If pathology has been excluded, time or change to another OC may solve the problem. Changing to an OC with a higher estrogen content, while potentially useful in minimizing menstrual irregularity, should be done only if necessary, since this may increase risk of thromboembolic disease. Women with past history of oligomenorrhea or secondary amenorrhea or young women without regular cycles may have a tendency to remain anovulatory or to become amenorrheic after discontinuing OC's. Women with these pre-existing problems should be advised of this possibility and encouraged to use other methods. Post-use anovulation, possibly prolonged, may also occur in women without previous irregularities.

11. **Ectopic Pregnancy**—Ectopic as well as intrauterine pregnancy may occur in contraceptive failures.

12. **Breast-feeding**—OC's given in the postpartum period may interfere with lactation and decrease quantity and quality of breast milk. Furthermore, a small fraction of the hormones in OC's has been identified in the milk of mothers on OC's; effects, if any, on the breast-fed child have not been determined. If feasible, defer OC's until infant has been weaned.

**Precautions**—GENERAL—1. A complete medical and family history should be taken prior to initiation of OC's. Pretreatment and periodic physical examinations should include special reference to blood pressure, breasts, abdomen and pelvic organs, including Pap smear and relevant laboratory tests. As a general rule OC's should not be prescribed for longer than 1 year without another physical examination.

2. Under influence of estrogen-progestogen preparations, pre-existing uterine leiomyomata may increase in size.

3. Patients with history of psychic depression should be carefully observed and the drug discontinued if depression recurs to a serious degree. Patients becoming significantly depressed while on OC's should stop OC's and use an alternate method to try to determine whether the symptom is drug-related.

4. OC's may cause some degree of fluid retention. They should be prescribed with caution, and only with careful monitoring, in patients with conditions which might be aggravated by fluid retention, such as convulsive disorders, migraine syndrome, asthma, or cardiac or renal insufficiency.

5. Patients with a past history of jaundice during pregnancy have an increased risk of recurrence while on OC's. If jaundice develops, OC's should be discontinued.

6. Steroid hormones may be poorly metabolized in patients with impaired liver function and should be administered with caution.

7. OC users may have disturbances in normal tryptophan metabolism which may result in a relative pyridoxine deficiency. Clinical significance is undetermined.

8. Serum folate levels may be depressed by OC's. Since the pregnant woman is predisposed to development of folate deficiency and incidence of folate deficiency increases with increasing gestation, it is possible that if a woman becomes pregnant shortly after stopping OC's, she may have a greater chance of developing folate deficiency and complications attributed to this deficiency.

9. The pathologist should be advised of OC therapy when relevant specimens are submitted.

10. Certain endocrine- and liver-function tests and blood components may be affected by estrogen-containing OC's:

a. Increased sulfobromophthalen retention. b. Increased prothrombin and factors VII, VIII, IX, and X; decreased antithrombin. c. Increased norepinephrine-induced platelet aggregability. d. Increased thyroid-binding globulin (TBG) leading to increased circulating total-thyroid hormone, as measured by protein-bound iodine (PBI), T4 by column, or T4 by radioimmunoassay. Free T3 resin uptake is decreased, reflecting the elevated TBG; free T4 concentration is unaltered. e. Decreased pregnanediol excretion. f. Reduced response to metyrapone test.

**Information for the Patient**—See Patient Package Labeling.

**Drug Interactions**—Reduced efficacy and increased incidence of breakthrough bleeding have been associated with concomitant use of rifampin. A similar association has been suggested with barbiturates, phenylbutazone, phenytoin sodium, ampicillin and tetracycline.

**Carcinogenesis**—See Warnings on carcinogenic potential of OC's.

**Pregnancy**—Category X. See Contraindications, Warnings.

**Nursing Mothers**—See Warnings.

**Adverse Reactions**—An increased risk of these serious adverse reactions has been associated with use of OC's (see Warnings): thrombophlebitis, pulmonary embolism, coronary thrombosis, cerebral thrombosis, cerebral hemorrhage, hypertension, gallbladder disease, benign hepatomas, congenital anomalies. There is evidence of an association between the following conditions and use of OC's although additional confirmatory studies are needed: mesenteric thrombosis, neuro-ocular lesions, e.g., retinal thrombosis and optic neuritis.

The following adverse reactions have been reported in patients on OC's and are believed to be drug-related. Nausea and/or vomiting, usually the most common adverse reactions, occur in approximately 10 percent or less of patients during the first cycle. Other reactions, as a general rule, are seen much less frequently or only occasionally. Gastrointestinal symptoms (such as abdominal cramps and bloating); breakthrough bleeding, spotting, change in menstrual flow, dysmenorrhea, amenorrhea during and after treatment, temporary infertility after discontinuance of treatment; edema; chloasma or melasma which may persist; breast changes: tenderness, enlargement, and secretion; change in weight (increase or decrease); change in cervical erosion and cervical secretion; possible diminution in lactation when given immediately postpartum; cholestatic jaundice; migraine; increase in size of uterine leiomyomata; rash (allergic); mental depression; reduced tolerance to carbohydrates; vaginal candidiasis; change in corneal curvature (steepening); intolerance to contact lenses.

The following adverse reactions have been reported in users of OC's, and the association has been neither confirmed nor refuted: premenstrual-like syndrome, cataracts, changes in libido, chorea, changes in appetite, cystitis-like syndrome, headache, nervousness, dizziness, hirsutism, loss of scalp hair, erythema multiforme, erythema nodosum, hemorrhagic eruption, vaginitis, porphyria.

**Acute Overdose**—Serious ill effects have not been reported following acute ingestion of large doses of OC's by young children. Overdose may cause nausea, and withdrawal bleeding may occur in females.

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Patterns of fetal lamb regional cerebral blood flow  
during and after prolonged hypoxia: Studies during  
the posthypoxic recovery period

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In an effort to determine to what extent cerebral blood flow (CBF) varies in different parts of the brain after prolonged fetal hypoxia, we measured flow to 34 regions. In seven chronically catheterized fetal lambs at 130 to 140 days' gestation, flow was measured by means of radioactive labeled microspheres during a control period, during hypoxia, and at 4, 24, and 48 hours after hypoxia: Control blood flow to cortical, subcortical, and brain stem structures respectively equaled 221, 237 and 275 ml · min<sup>-1</sup> · 100 gm<sup>-1</sup>. After 90 minutes of hypoxia, flows respectively increased 67%, 93%, and 160% to these areas, suggesting preferential shunting of flow to critical brain stem regions. By 4, 24, and 48 hours after hypoxia the regional flows had returned to values not significantly different from control values. We conclude that: (1) significant fetal regional cerebral blood flow differences occurred in utero, with brain stem and subcortical flows being greater than flows to other regions of the brain; (2) during prolonged intrauterine hypoxia, total cerebral blood flow increased about 95%, with evidence of preferential shunting to critical brain stem regions; (3) during the posthypoxic recovery period at 4, 24, and 48 hours, flows returned to normal, indicating no evidence of a post-ischemic hypoperfusion syndrome or impairment of the microcirculation. (AM. J. OBSTET. GYNECOL. 139:365, 1981.)

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INTRAUTERINE HYPOXIA associated with fetal distress constitutes one of the most important problems of pregnancy. In various mammalian species, periods of partial to total asphyxia produce significant elevations of cerebral blood flow (CBF) that endure for the duration of the hypoxic episode.<sup>7</sup> Recovery blood flow values are dependent on the duration and magnitude of hypoxia and the degree of acidosis. It has been suggested that, following a hypoxic-ischemic episode, fetal CBF decreases markedly with preferential shunting to

Table I. Fetal CBF during and after hypoxia

| Area         | Control<br>(ml · min · 100 gm <sup>-1</sup> ) | Change at 90 minutes of hypoxia<br>(ml · min · 100 gm <sup>-1</sup> ) |         | Change at 4 hours after hypoxia<br>(ml · min · 100 gm <sup>-1</sup> ) |         |
|--------------|-----------------------------------------------|-----------------------------------------------------------------------|---------|-----------------------------------------------------------------------|---------|
|              |                                               | Mean                                                                  | Percent | Mean                                                                  | Percent |
| Total brain  | 208.3 (±36.7)                                 | ±8.3 (±56.0)                                                          | +95.9*  | 235.8 (±43.5)                                                         | +9.1    |
| Cortex       | 221.2 (±74.7)                                 | ±0.0 (±90.1)                                                          | +67.3*  | 238.5 (±66.8)                                                         | +0.2    |
| Gray matter  | 243.1 (±76.1)                                 | ±6.7 (±92.4)                                                          | +67.3*  | 240.6 (±68.9)                                                         | -7.4    |
| White matter | 183.0 (±71.4)                                 | ±2.2 (±87.3)                                                          | +76.1*  | 238.2 (±63.1)                                                         | +22.1   |
| Subcortex    | 237.4 (±60.0)                                 | ±9.1 (±81.8)                                                          | +93.3*  | 300.7 (±60.9)                                                         | +22.6   |
| Brain stem   | 275.1 (±89.9)                                 | ±4.8 (±156.7)                                                         | +156.2* | 377.7 (±78.6)                                                         | +30.5   |

\*Significance: P < 0.01.

Table II. Organ blood flows

| Organ          | Control<br>(ml · min · 100 gm <sup>-1</sup> ) | Change at 90 minutes of hypoxia<br>(ml · min · 100 gm <sup>-1</sup> ) |         | Change at 4 hours after hypoxia<br>(ml · min · 100 gm <sup>-1</sup> ) |         |
|----------------|-----------------------------------------------|-----------------------------------------------------------------------|---------|-----------------------------------------------------------------------|---------|
|                |                                               | Mean                                                                  | Percent | Mean                                                                  | Percent |
| Brain          | 208.3 (±36.7)                                 | -08.3 (±56.0)                                                         | +95.9*  | 235.8 (±43.5)                                                         | +9.9    |
| Heart          | 220.6 (±24.5)                                 | ±38.3 (±75.5)                                                         | +279.9* | 367.0 (±59.8)                                                         | +61.8†  |
| Lungs          | 405.3 (±104.2)                                | ±89.3 (±97.0)                                                         | -28.6   | 128.9 (±38.9)                                                         | -68.1   |
| Kidneys        | 151.7 (±18.2)                                 | 91.5 (±22.5)                                                          | -39.7*  | 201.0 (±41.6)                                                         | +35.6   |
| Adrenal glands | 189.9 (±33.5)                                 | ±69.7 (±60.7)                                                         | +410.6* | 631.7 (±138.6)                                                        | +228.6† |
| Placenta       | 126.2 (±13.8)                                 | ±27.2 (±15.8)                                                         | +0.9    | 103.8 (±15.4)                                                         | -15.4   |

\*Significance: p < 0.01.

†Significance: p < 0.05.

some parts of the fetal brain.<sup>10</sup> It is this secondary cerebral hypoperfusion caused by the development of diffuse cerebral edema and impairment of the microcirculation that has been postulated to contribute significantly to brain injury.<sup>6</sup> In adult dogs total cerebral ischemia for 15 minutes is followed by markedly increased intracranial pressure and a threefold increase in cortical blood flow.<sup>11</sup> Forty minutes after restoration of the cerebral circulation, CBF falls to 50% of the control value despite a normalization of intracranial pressure, and during this time cerebral oxygen consumption and glucose metabolism remain well below control values. In gerbils, unilateral or bilateral carotid occlusion for 1 hour results in an altered to absent electroencephalogram. Following the restoration of cerebral circulation the flow increases for 5 to 10 minutes, followed by a decrease to 50% of the control value despite an increase in cerebral metabolic rate.<sup>6</sup>

No previous studies have examined the changes which occur in fetal CBF for prolonged time periods after a hypoxic insult. Therefore, in an effort to understand further the effects of prolonged intrauterine hypoxia we have determined to what extent regional CBFs change during prolonged intrauterine hypoxia as well as during a 48-hour recovery period.

Material and methods

We studied seven ewes of 120 to 140 days' gestation and their fetuses. We anesthetized the ewe with 2 ml of 0.2% tetracaine hydrochloride given intraspinally, in addition to 400 mg of sodium pentobarbital intravenously. We placed Tygon catheters (2.3 mm outside diameter) in a branch of the maternal popliteal artery. Through a small hysterectomy incision, we extracted a fetal hind limb. Following the injection of local anesthesia (1 ml of 0.5% lidocaine), we placed catheters (1.8 mm outside diameter) into both a dorsal pedal artery and vein and advanced the tips respectively into the descending aorta and inferior vena cava below the ductus venosus. Through a separate small incision we also extracted a fetal forelimb and, after anesthetizing the skin, placed two catheters, one from the radial artery into the brachiocephalic artery or the ascending aorta and one from the cephalic vein into the superior vena cava. In addition, we positioned an amniotic fluid catheter over the left chest wall. Following closure in layers, we exteriorized the fetal and maternal catheters to the ewe's flank and allowed the ewe to recover.

Five or six days after operation, we carried out the experiments. We recorded control fetal and maternal blood pressures and heart rates. In addition, we ob-



| Change at 24 hours after hypoxia<br>(ml · min · 100 gm <sup>-1</sup> ) |         | Change at 48 hours after hypoxia<br>(ml · min · 100 gm <sup>-1</sup> ) |         |
|------------------------------------------------------------------------|---------|------------------------------------------------------------------------|---------|
| Mean                                                                   | Percent | Mean                                                                   | Percent |
| 220.0 (±30.7)                                                          | +1.2    | 235.8 (±14.7)                                                          | +7.0    |
| 226.3 (±64.5)                                                          | -5.0    | 272.9 (±51.2)                                                          | +16.0   |
| 238.1 (±78.0)                                                          | -8.4    | 295.5 (±64.7)                                                          | +14.1   |
| 205.8 (±45.1)                                                          | +4.3    | 239.2 (±36.2)                                                          | +23.6   |
| 278.4 (±67.4)                                                          | +13.2   | 300.5 (±26.4)                                                          | +22.5   |
| 339.3 (±90.1)                                                          | +16.6   | 378.6 (±37.2)                                                          | +32.1   |

| Change at 24 hours after hypoxia<br>(ml · min · 100 gm <sup>-1</sup> ) |         | Change at 48 hours after hypoxia<br>(ml · min · 100 gm <sup>-1</sup> ) |         |
|------------------------------------------------------------------------|---------|------------------------------------------------------------------------|---------|
| Mean                                                                   | Percent | Mean                                                                   | Percent |
| 220.0 (±30.7)                                                          | +1.2    | 235.8 (±14.7)                                                          | +7.0    |
| 306.3 (±28.6)                                                          | +34.2*  | 487.3 (±143.9)                                                         | +112.9  |
| 423.0 (±108.9)                                                         | +4.4    | 627.1 (±156.9)                                                         | +46.5   |
| 172.3 (±11.9)                                                          | +16.7   | 212.6 (±38.9)                                                          | +34.3   |
| 338.0 (±60.6)                                                          | 73.9    | 574.3 (±141.1)                                                         | 205.2†  |
| 113.8 (±11.7)                                                          | -7.5    | 92.2 (±11.9)                                                           | -31.0†  |

tained anaerobic heparinized blood samples from the maternal artery, fetal ascending and descending aortas, and superior and inferior venae cavae for measurement of oxygen and carbon dioxide tensions and pH, with appropriate microelectrodes (Radiometer ABL 2, London Company, Westlake, Ohio), and the hematocrit.

In order to determine blood flows to the various regions of the brain and other organs, we used microspheres 15 (±1) μ in diameter labeled with <sup>57</sup>Co, <sup>51</sup>Cr, <sup>113</sup>Sn, <sup>85</sup>Sr, or <sup>46</sup>Sc (Minnesota Mining and Manufacturing Co., St. Paul, Minnesota). These were suspended in a solution of 10% dextran and 0.05% polyoxyethylene sorbitan monooleate (Tween 80) to minimize aggregation and provide for even distribution. Prior to the experiment, we placed vials containing microspheres into an ultrasonic bath for 10 to 30 minutes for dispersion. To measure control blood flows, we injected the microspheres over a 15-second period into the inferior (two thirds) and superior (one third) vena cava. During the period of injection and for 75 seconds thereafter, we withdrew blood reference samples at a rate of 4.8 ml · min<sup>-1</sup> simultaneously from both the ascending and the descending aorta, by means of an infusion-withdrawal pump (Model 972, Harvard Apparatus Company, Inc., Millis, Maine).

After placing the ewe's head in a plastic hood, we induced hypoxia by having the ewe inspire 9% to 10%

oxygen and 5% carbon dioxide to offset the respiratory alkalosis induced by hyperventilation, at a gas flow rate of 15 · L · min<sup>-1</sup>. We sampled blood from the fetal ascending aorta every 5 minutes until the oxygen tension equaled 12 to 15 torr. This was considered our zero time reference for institution of hypoxia. At 90 minutes after the onset of hypoxia, we injected radio-nuclide-labeled microspheres. We obtained blood for complete blood gas determinations prior to the microsphere injection and restored fetal blood volume with an equal volume of maternal blood. Occasionally we administered 3 to 5 ml of sodium bicarbonate in order to correct the metabolic acidosis induced by hypoxia. At the end of the 90-minute hypoxic period, we removed the hood and allowed the ewe to breathe room air and recover. At 4, 24, and 48 hours after hypoxia we injected microspheres labeled with different radionuclides and obtained blood gas determinations and monitored fetal and maternal cardiovascular functions.

The ewe and fetus were then killed with 30 ml of T61 (National Laboratories Corp., Summerville, New Jersey). The fetus was removed, towel dried, and weighed, and the major fetal organs (heart, lungs, liver, gastrointestinal tract, kidneys, adrenals, and spleen) as well as the placental cotyledons and amniotic membranes were removed, weighed, ashed, and reweighed. We dissected the fetal brain into 34 discrete regions, including the following: cortical structures—the gray and white matter of the frontal, parietal, temporal, and occipital lobes; subcortical structures—thalamus, hypothalamus, caudate nucleus, hippocampus, and cerebellum; brain stem structures—midbrain, pons, and medulla; and the cervical spinal cord. These were weighed separately and placed into plastic-covered vials. Radioactivity was determined in all samples (Auto-Gamma Scintillation Spectrometer, Model 5912, Packard Instrument Company, Inc., Downers Grove, Illinois). Specimens from the brain weighed approximately 5 gm and contained 400 to 800 microspheres. By means of a digital computer, we calculated the quantity of microspheres per organ and then converted the counts to absolute blood flows, expressed as ml · min<sup>-1</sup> · 100 gm organ weight<sup>-1</sup>, ml · min<sup>-1</sup> · kg fetus<sup>-1</sup>, relative flow, and percent of cardiac output.

For each variable under consideration we performed statistical calculations on the control measurements, the experimental measurements, and the paired differences between experimental and control values. We computed mean values, standard deviations, and standard errors of the means and used the paired t test to determine the significance of changes from control.

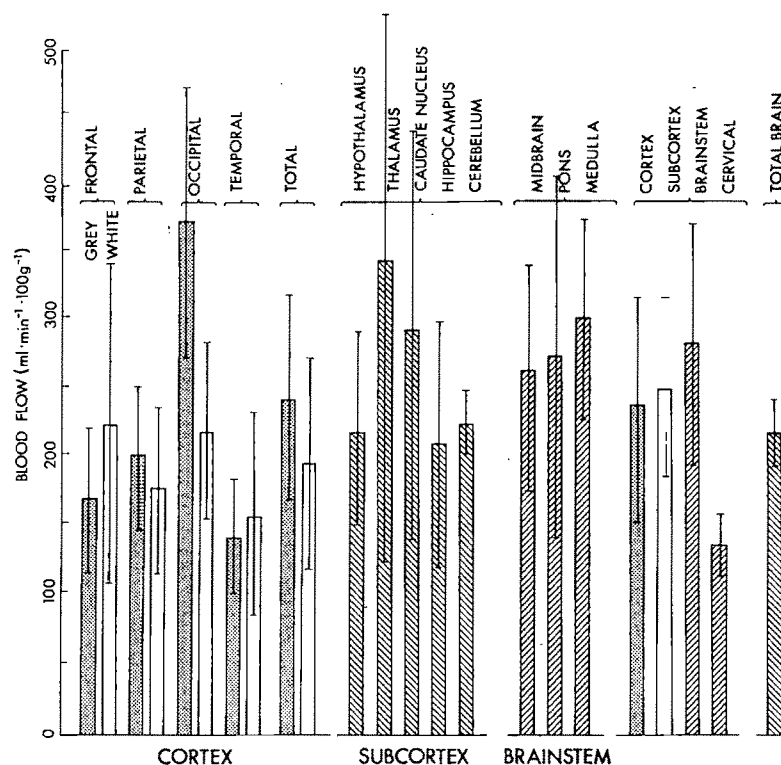


Fig. 1. Control total and regional fetal CBF ( $\text{ml} \cdot \text{min}^{-1} \cdot 100 \text{ gm}^{-1}$ ) to discrete regions of the brain. Regional values are based on combined data for both right and left sides of brain. The values are means ( $\pm \text{SEM}$ ) from seven lambs.

## Results

**Control total and regional fetal CBF.** Total fetal CBF equaled  $208.3 (\pm 36.7 \text{ SEM}) \text{ ml} \cdot \text{min}^{-1} \cdot 100 \text{ gm brain tissue}^{-1}$  (Fig 1, Table I). Flow was not uniform to all parts of the brain, however, and Fig. 1 shows the hierarchy of blood flow with an increase from a cephalad to a caudad direction. Thus, the mean cortical blood flow equaled  $221.2 (\pm 74.7) \text{ ml} \cdot \text{min}^{-1} \cdot 100 \text{ gm}^{-1}$ , the subcortical flow was  $237.4 (\pm 60.0) \text{ ml} \cdot \text{min}^{-1} \cdot 100 \text{ gm}^{-1}$ , and the brain stem flow was  $275.1 (\pm 89.9) \text{ ml} \cdot \text{min}^{-1} \cdot 100 \text{ gm}^{-1}$  (Table I). There was a surprising consistency of similar blood flows to various structures within the major regions, except for a mean cortical gray matter flow of  $243.1 (\pm 76.1) \text{ ml} \cdot \text{min}^{-1} \cdot 100 \text{ gm}^{-1}$  as compared to a white matter blood flow of  $183.0 (\pm 71.4) \text{ ml} \cdot \text{min}^{-1} \cdot 100 \text{ gm}^{-1}$ .

**Fetal blood gases and cardiovascular response during and after hypoxia.** Fig. 2 depicts the changes in oxygen and carbon dioxide tensions in the fetal ascending and descending aorta and the pH during and after the 90-minute hypoxia. During the entire hypoxic period, the ascending aortic oxygen tension was maintained at 12 to 15 torr while the carbon dioxide tension remained relatively constant at 48 torr. Fetal pH de-

creased from a control value of  $7.34 (\pm 0.01)$  to  $7.22 (\pm 0.01)$  at 90 minutes. During the recovery phase, these values returned to the control values by 4 hours and remained at these levels at 24 and 48 hours.

Fig. 3 shows that the fetal mean arterial blood pressure was 49 mm Hg and did not vary significantly during the experiment. The control fetal heart rate was 162 bpm, and during hypoxia it increased slightly. By 4 hours after hypoxia it had increased 20% above control to 194 bpm, after which it steadily decreased. At 90 minutes of hypoxia the cardiac output remained relatively constant at  $727 (\pm 64) \text{ ml} \cdot \text{min}^{-1} \cdot \text{kg fetal weight}^{-1}$ ; however, the fraction of cardiac output to the brain doubled to  $9.2 (\pm 0.6\%)$  from  $4.2 (\pm 0.8\%)$ .

**Changes in regional cerebral blood flow during hypoxia.** As shown in Fig. 4, after 90 minutes of hypoxia, blood flow to the brain stem increased to  $704.8 (\pm 156.7)$  from  $275.1 (\pm 89.9) \text{ ml} \cdot \text{min}^{-1} \cdot 100 \text{ gm}^{-1}$ , flow to subcortical regions increased to  $459.1 (\pm 81.8)$  from  $237.4 (\pm 60.0) \text{ ml} \cdot \text{min}^{-1} \cdot 100 \text{ gm}^{-1}$ , and flow to the cortex increased to  $370 (\pm 90.1)$  from  $221.2 (\pm 74.7) \text{ ml} \cdot \text{min} \cdot 100 \text{ gm}^{-1}$ . Table I shows the mean changes ( $\pm \text{SEM}$ ) in absolute flow at 90 minutes of hypoxia and during the recovery period at 4, 24,



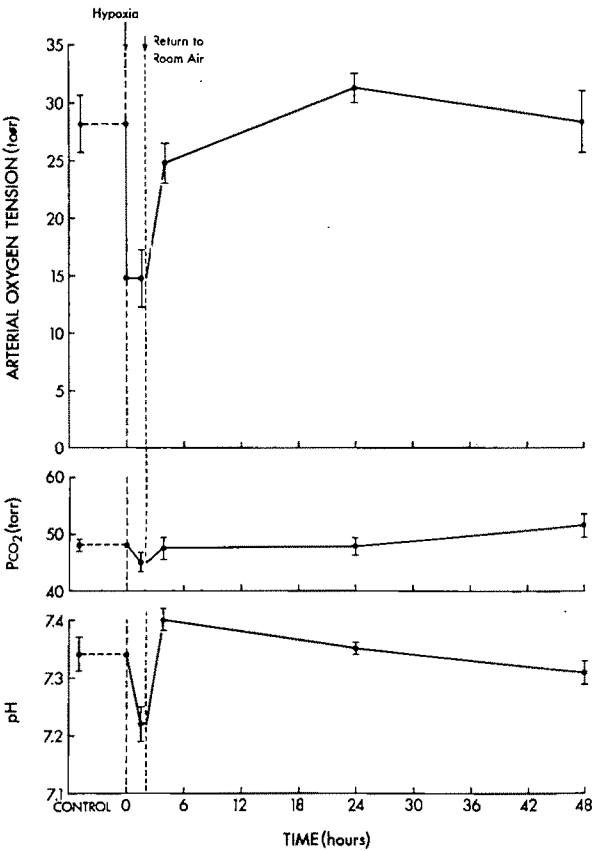


Fig. 2. Fetal ascending aortic  $\text{Po}_2$ , oxygen content,  $\text{PCO}_2$ , and pH before hypoxia, during the 90-minute hypoxic period, and during the recovery period at 4, 24, and 48 hours. Mean values  $\pm$  SEM.

and 48 hours. In all regions, flow at 4 hours after hypoxia did not differ significantly from control values and remained constant at 24 and 48 hours (Table I and Fig. 4.)

Fig. 5 shows the percent increase in blood flow to the major regions of the brain and demonstrates a preferential increase of blood flow to the brain stem. For instance, flow to the brain stem increased 156% above control values at 90 minutes as compared to the 67% increase in the cortex and the 93% increase in the subcortex. However, we observed no evidence of preferential shunting of blood flow to certain brain regions during the recovery periods.

**Changes in organ blood flow during and after hypoxia.** During hypoxia, blood flow to the heart and adrenal glands also increased and remained elevated during the entire 48-hour recovery period. Blood flows to the lungs and kidneys decreased during hypoxia but returned to values slightly higher than control levels by 48 hours. Table II shows the mean changes in flow

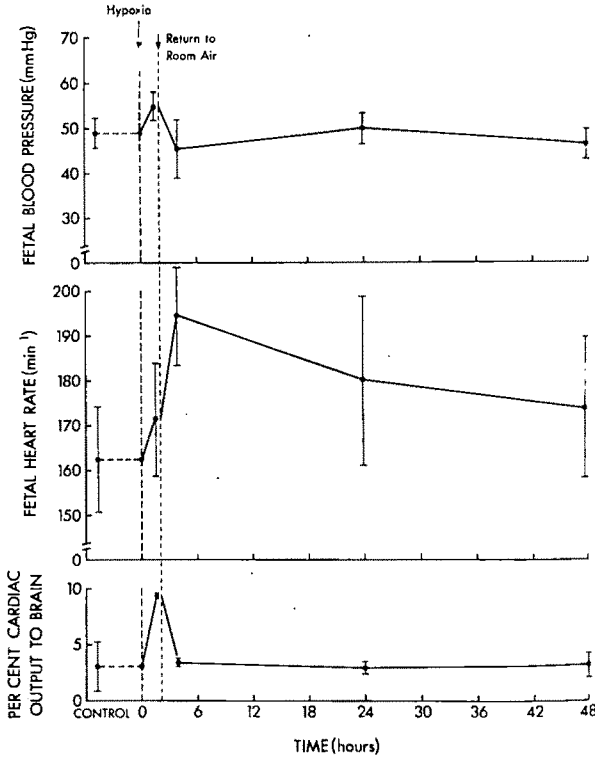


Fig. 3. Mean arterial blood pressure, heart rate, and percent cardiac output to brain before hypoxia, during the 90-minute hypoxic period, and during the recovery period at 4, 24, and 48 hours. Mean values  $\pm$  SEM.

during and after the hypoxic period as both an absolute value and a percent change.

Comment

**Control regional CBF.** The control value of total cerebral flow of  $208.3 (\pm 36.7) \text{ ml} \cdot \text{min}^{-1} \cdot 100 \text{ gm}^{-1}$  found in this study (Fig. 1 and Table I) is somewhat higher than our previous value of  $155 (\pm 16) \text{ ml} \cdot \text{min}^{-1} \cdot 100 \text{ gm}^{-1}$ . We suspect that this resulted primarily from both a higher mean cardiac output of  $727 (\pm 64) \text{ ml} \cdot \text{min}^{-1} \cdot \text{kg}$  fetal weight<sup>-1</sup> as compared to a value of  $551 (\pm 38) \text{ ml} \cdot \text{min}^{-1} \cdot \text{kg}^{-1}$  and a higher mean  $\text{PCO}_2$  of 48 torr in this group as compared to a mean value of 42 torr in our previous study.

The hierarchy of control regional blood flows observed in this study confirms our previous finding of the increase in CBF in progression from a cephalad to a caudad direction (Table I), the mean flows of the cortex, subcortex, and brain stem respectively equaling 221.2, 237.4, and 275.1  $\text{ml} \cdot \text{min}^{-1} \cdot 100 \text{ gm}^{-1}$ . There was a surprising consistency of blood flows to various structures within the major regions except for an elevation of mean cortical gray matter flow (to 243.1) as

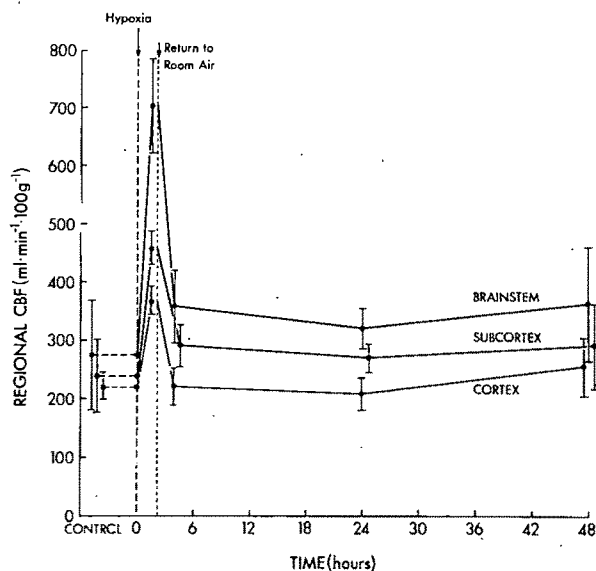


Fig. 4. Changes in total and regional fetal cerebral blood flow ( $\text{ml} \cdot \text{min}^{-1} \cdot 100 \text{ gm}^{-1}$ ) to the cortex, subcortex, and brain stem during the 90-minute hypoxic period and in the recovery period at 4, 24, and 48 hours. Values are means  $\pm$  SEM.

compared to mean white matter flow ( $183 \text{ ml} \cdot \text{min}^{-1} \cdot 100 \text{ gm}^{-1}$ ).

**Changes in regional CBF during hypoxia.** After a 90-minute period of moderate hypoxic stress in which the mean oxygen tension decreased to 15 from 28 torr, total and regional CBFs increased significantly above control values (Fig. 4.) Thus, total cerebral flow increased 96%, while the increases for the cortex, subcortex, and brain stem respectively were 93%, 67%, and 156%. These values differ from those of our previous study in which flows to the cortex and brain stem respectively increased 99% and 126%. We interpreted these later findings as showing that the brain's circulatory response to hypoxia is a generalized phenomenon, that is, all areas show a similar increase in flow. In this study, the percent increase in flow to the brain stem was twice that to the cortex, suggesting the possibility of preferential shunting of blood to these critical regions of the nervous system during hypoxic stress. This preferential shunting would serve as an important mechanism in the fetus and might explain previous neuropathologic studies by other investigators which demonstrated preservation of brain stem structures in the fetal monkey during periods of "partial prolonged intrauterine asphyxia."<sup>3</sup> It is still possible that an alternative adaptive protective mechanism associated with differences in fetal regional brain metabolism exists, and further studies of both regional CBF and metabolism are necessary to answer these critical questions.

In premature infants, subependymal and intraven-

tricular hemorrhage have been documented in more than 40% of cases by computerized tomography.<sup>9</sup> It has been suggested that hypoxic-ischemic injury to the germinal matrix periventricular region predisposes these areas to hemorrhage.<sup>12</sup> Thus, when the blood pressure of the newborn infant is restored to normal or above normal values or when hypercarbia develops because of severe hyaline membrane disease, CBF may increase substantially and the increased perfusion in the periventricular region could result in local hemorrhage.<sup>5</sup> Our studies in the fetus have shown no significant shunting of CBF to subcortical periventricular structures such as the caudate nucleus, hypothalamus, or thalamus. Thus, if hyperperfusion of the periventricular region does occur and is contributory to the development of intraventricular hemorrhage, these studies suggest that this phenomenon is associated with a generalized increase in CBF and not specific regional differences.

**Changes in regional CBF after hypoxia.** We determined total and regional CBFs at 4, 24, and 48 hours after the 90-minute period of hypoxic stress. By 4 hours all flows had returned to values slightly but not significantly different from control values (Fig. 4). At 24 and 48 hours similar values of flow to the total brain and all regions were observed. These findings are of interest in lieu of previous work in which postischemic hypoperfusion developed after varying periods of total cerebral ischemia, produced in dogs by aortic crossclamping or in gerbils by carotid artery occlusion.<sup>6, 11</sup> In these studies, after cerebral circulation was restored, CBF substantially increased, but, after 5 to 40 minutes, flows decreased to less than 50% of control values, where they persisted for several hours. The mechanism of this phenomenon is not well understood although local tissue alkalosis,<sup>6</sup> neurogenic factors associated with the release of vasoactive substances,<sup>13</sup> cerebral edema formation with impingement on the microcirculation,<sup>4</sup> and a marked imbalance between energy metabolism and flow<sup>6</sup> have all been postulated. It has also been suggested that this postischemic hypoperfusion syndrome may be a significant factor in further neuronal damage to tissue which still might be functional and viable. Cerebral edema can occur in infants who have had significant intrauterine and perinatal asphyxia. It has been suggested by some investigators that the development of edema can severely impair the cerebral microcirculation and cause significant brain damage. Based on this hypothesis, use of cerebral dehydrating agents such as corticosteroids or osmotic diuretics have been recommended as part of the acute management of perinatal asphyxia.<sup>2, 8</sup>

In this study of moderate to severe prolonged in-



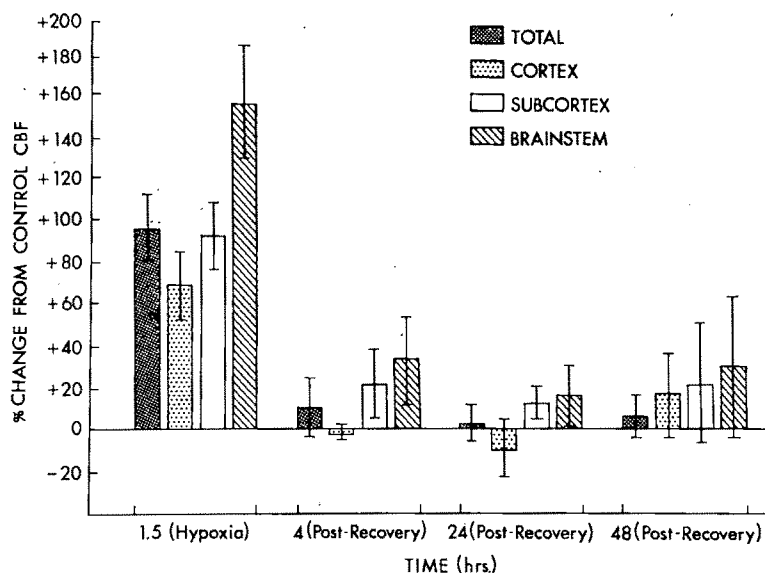


Fig. 5. Percent increase above control values in total and regional CBF during the hypoxic and recovery periods. Values are means  $\pm$  SEM.

trauterine hypoxic stress, we observed no evidence of a hypoperfusion syndrome at 4, 24, and 48 hours after hypoxia. Flows to all regions of the brain remained slightly greater than control values, indicating no significant impairment of the microcirculation. If these data can be applied to neonates, damage to the nervous system in infants who experience perinatal asphyxia presumably results from the hypoxic neuronal insult rather than from a significant impairment in cerebral blood flow. It may be that cerebral edema develops in the severely asphyxiated infant, but whether this can affect cerebral flow remains to be determined.

In several of the animals studied, cerebral flow also was determined in the "watershed" areas of the brain. These areas, representing the terminal collateral circulation between major cerebral blood vessels (such as the anterior and middle cerebral artery), are thought to be quite susceptible because of decreased cerebral perfusion associated with hypotension and cortical ischemia. During hypoxic stress without significant alteration in systemic blood pressure or cardiac output, flow to

watershed areas was similar to that in other main regions. These data are supportive evidence of the concept developed by other investigators that the combination of both hypoxic and hypotensive stress is responsible for watershed territorial ischemia.

In summary, in our studies of fetal CBF during and after prolonged hypoxia, we have noted the following: (1) Significant differences in blood flow to various regions of the fetal brain exist, with blood flow to the brain stem approximately twice that to the cortex and with subcortical structures receiving an intermediate flow. (2) During prolonged intrauterine hypoxia, total and regional blood flow increased 70% to 160% with evidence to suggest preferential shunting to critical brain stem regions. (3) By 4 hours after normal oxygenation is restored, total and regional CBFs have returned to control values. Up to 48 hours after hypoxia flow remains normal, with no preferential shunting to parts of the brain, indicating a preservation of the cerebral microcirculation.

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# Initiation of human parturition

## XII. Biosynthesis and metabolism of prostaglandins in human fetal membranes and uterine decidua

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Prostaglandin synthetase activity was assayed in homogenates prepared from human fetal membranes and uterine decidua tissues with [ $^{14}\text{C}$ ]arachidonic acid used as substrate. In amnion, chorion laeve, and decidua vera tissues obtained after the spontaneous onset of labor and vaginal delivery the mean specific activities of prostaglandin synthetase were respectively 1.47, 0.53, and 0.39 nmoles  $\times$  hr $^{-1}$   $\times$  gm $^{-1}$  tissue; in these same tissues obtained at elective cesarean section before the onset of labor the mean specific activities of prostaglandin synthetase were respectively 0.61, 0.33, and 0.28. The specific activity of prostaglandin synthetase of amnion (but not chorion laeve or decidua vera) was significantly greater in samples obtained after labor than in those obtained before labor ( $p < 0.02$ ). Prostaglandin synthesis in amnion, chorion laeve, and decidua vera was inhibited by acetylsalicylic acid and by indomethacin. Prostaglandin  $\text{E}_2$  was formed in amnion and chorion laeve, whereas prostaglandin  $\text{E}_2$  and prostaglandin  $\text{F}_{2\alpha}$  were formed in decidua vera. The activity of  $\text{NAD}^+$ -dependent 15-hydroxyprostaglandin dehydrogenase was also assayed and the specific activities of this enzyme in chorion laeve and decidua vera tissues obtained before and after labor were high, whereas 15-hydroxyprostaglandin dehydrogenase activity in amnion was low. (AM. J. OBSTET. GYNECOL. 139:373, 1981.)

THE FETAL MEMBRANES and uterine decidua vera are believed to have a central role in the metabolic events that lead to the initiation of parturition in women.<sup>1</sup> Considerable evidence also has accrued which is supportive of the view that prostaglandins, or chemically related substances, which are produced in these

tissues act directly on the myometrium to initiate labor.<sup>1,2</sup> After the onset of labor and throughout active labor, the levels of prostaglandin  $\text{E}_2$  ( $\text{PGE}_2$ ), prostaglandin  $\text{F}_{2\alpha}$  ( $\text{PGF}_{2\alpha}$ ), and/or metabolites thereof are elevated in amniotic fluid,<sup>3-5</sup> maternal plasma,<sup>4-6</sup> and urine<sup>4,7</sup> compared to the levels of these substances before labor commences.

The level of free arachidonic acid, the obligate precursor of prostaglandins of the 2-series, is also increased (and disproportionately so compared to other free fatty acids) in the amniotic fluid of women in labor compared to that in amniotic fluid of women not in labor<sup>8</sup>; moreover, the levels of arachidonic acid in amniotic fluid continue to increase as cervical dilatation advances.<sup>9</sup> In a previous investigation we found that human fetal membranes (amnion and chorion laeve) were enriched in arachidonic acid.<sup>10</sup> The lipid species of the fetal membranes that is most enriched in arachidonic acid is phosphatidylethanolamine, which constitutes only 30% of the total glycerophospholipids

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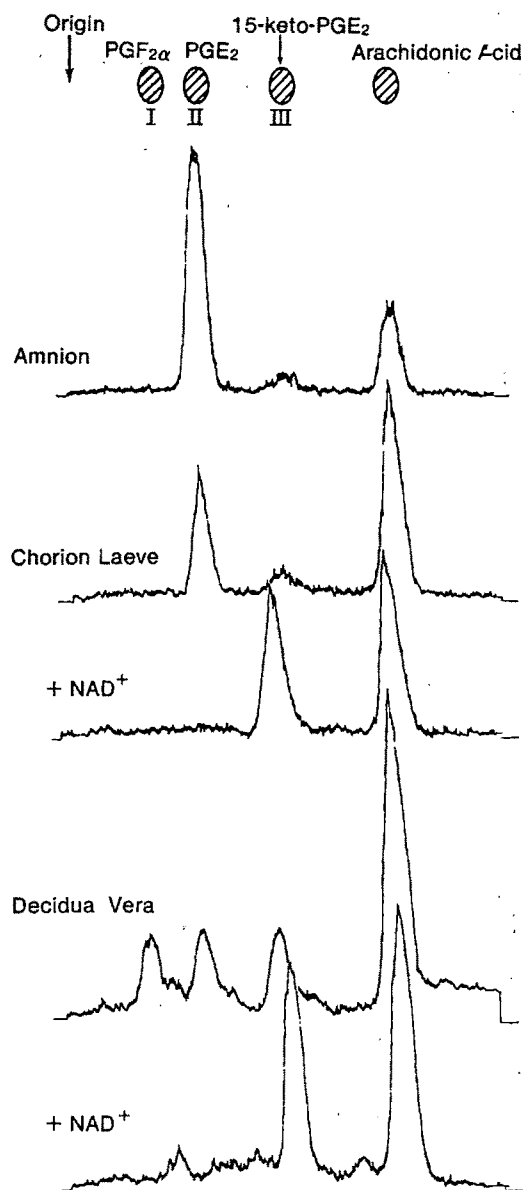


**Fig. 1.** Radiochromatography scan of radioactive prostaglandin products formed in homogenates of human amnion, chorion laeve, and uterine decidua vera tissues incubated with [ $^{14}\text{C}$ ] arachidonic acid. The whole homogenate of either amnion, chorion laeve, or decidua vera tissue (0.5 gm wet weight of each tissue) was incubated with 5 nmol of [ $^{14}\text{C}$ ] arachidonic acid at 37°C for 10 minutes (amnion) or 20 minutes (chorion laeve and decidua vera) in the presence or absence of  $\text{NAD}^+$  (3 mM). The prostaglandin fraction was isolated by chromatography on Unisil columns, and an aliquot was analyzed by thin-layer chromatography with the use of solvent system A.  $\text{PGF}_{2\alpha}$ ,  $\text{PGE}_2$ , 15-keto- $\text{PGE}_2$ , and Arachidonic Acid refer to positions on the chromatogram of authentic standards which were chromatographed in parallel lanes.

yet contains 50% to 60% of the arachidonic acid found in the glycerophospholipids of these tissues.<sup>11</sup> The phospholipase  $\text{A}_2$  of human fetal membranes has substrate specificity for phosphatidylethanolamines and especially for *sn*-2-arachidonoyl esters of this glycerophospholipid, whereas no specificity for *sn*-2-arachidonoyl esters of phosphatidylethanolamines was found for the phospholipase  $\text{A}_2$  of uterine decidua vera tissue.<sup>12</sup> Taken together, these findings are supportive of the view that the human fetal membranes are an important site of release of free arachidonic acid during human labor.

Prostaglandins are found in high concentrations in uterine decidual tissues obtained from women in labor,<sup>13</sup> a finding which is suggestive that the prostaglandin(s) which is involved in the initiation of parturition in women is synthesized in the decidua vera. The decidua vera,<sup>14-16</sup> fetal membranes,<sup>14-17</sup> myometrium,<sup>14</sup> and umbilical vessels,<sup>18</sup> are known to contain prostaglandin synthetase activity. The presence of prostaglandin synthetase in these tissues was established by a number of investigators using a variety of techniques, e.g., radioimmunoassay of prostaglandins and metabolites in tissue superfusates<sup>15</sup> and the measurement of the rate of conversion of radiolabeled precursor fatty acids to radiolabeled prostaglandins.<sup>16, 17</sup> However, the results of systematic studies of prostaglandin synthetase and  $\text{NAD}^+$ -dependent 15-hydroxyprostaglandin dehydrogenase (PGDH) activities in these tissues have not been reported.

The present study was undertaken to evaluate the enzymatic activities involved in the biosynthesis and inactivation of prostaglandins in human fetal membranes and decidua vera tissues.

#### Material and methods

**Preparation of tissues.** Fetal membranes and attached decidua vera were removed from placentas collected at the time of spontaneous vaginal delivery or at the time of elective cesarean section performed prior to the onset of labor. The tissues were washed several times in an ice-cold solution of 0.15M NaCl. The amnion tissue was peeled away from the chorion laeve and the decidua vera was removed from the chorion laeve by sharp dissection. By means of this technique it was found, by histologic examination, that the chorion laeve, after dissection, was not contaminated with decidual tissue.

**Material.** [ $1\text{-}^{14}\text{C}$ ] Arachidonic acid (58 mCi/mmol), [5,6,8,11,12,14,15(n)- $^3\text{H}$ ]  $\text{PGE}_2$  (160 Ci/mmol) and [5,6,8,11,12,14,15(n)- $^3\text{H}$ ]  $\text{PGF}_{2\alpha}$  (150 Ci/mmol) were purchased from Amersham Corp., Arlington Heights,



Illinois. Nonradiolabeled arachidonic and oleic acids were obtained from Nu Chek Prep, Elysian, Minnesota; PGE<sub>2</sub>, hematin, reduced glutathione, L-tryptophan, indomethacin, and acetylsalicylic acid were purchased from Sigma Chemical Co., St. Louis, Missouri; and Unisil was obtained from Clarkson Chemical Co., Williamsport, Pennsylvania. All other chemicals were of reagent grade.

**Assay of prostaglandin synthetase activity.** The substrate employed for the prostaglandin synthetase assay, [<sup>14</sup>C]arachidonic acid, was purified before use by chromatography on a Unisil column. The chloroform washings of this column contained purified [<sup>14</sup>C]arachidonic acid; the chloroform was evaporated and the substrate was prepared as the sodium salt in a solution of 0.15M NaCl. The stock solution of substrate contained approximately 100 nmoles of [<sup>14</sup>C]arachidonic acid per milliliter. For the assay of prostaglandin synthetase activity, the tissues were cut into small pieces, and 0.5 gm wet weight of tissue was placed in 5.0 ml of potassium phosphate buffer (50 mM, pH 7.4) containing ethylenediaminetetra-acetate (EDTA) (2 mM) and homogenized in a Polytron PT10 homogenizer at a setting of 6 for 10 seconds. Prostaglandin synthetase assays were conducted by incubating the entire 5.5 ml of homogenate with [<sup>14</sup>C]arachidonic acid (5 nmoles), L-tryptophan (25 μmoles), reduced glutathione (31 μmoles), and hematin (10 nmoles) in a final volume of 6 ml at 37° C for various times. It has been established by numerous investigators that L-tryptophan and hematin are required for the formation of prostaglandin H<sub>2</sub> (PGH<sub>2</sub>) from arachidonic acid. In addition, reduced glutathione is required for formation of PGE<sub>2</sub> from PGH<sub>2</sub>. The cofactors were added to all incubation vessels in order that any changes in the type of prostaglandin formation that occurred under these conditions would be a reflection of the enzymes catalyzing the specific enzymatic steps rather than being due to the limitation of the availability of the cofactors.

Reactions were terminated by the addition of 20 ml of ethanol (95%) to the reaction mixture. After the ethanol-treated reaction mixture was filtered through Whatman No. 2 filter paper and the incubation flask and filter paper were washed with an additional 20 ml of ethanol, the filtrates were combined and the solvent was evaporated by means of a rotary evaporator. The residue was dissolved in 5.0 ml of water and acidified by the addition of 0.2 ml of 2M citric acid, and the lipids were extracted with 15 ml of chloroform:methanol (2:1, v:v). After centrifugation to partition the solvents, the aqueous layer was removed and the

**Table I.** Inhibition of PGE<sub>2</sub> formation in amnion, chorion laeve, and decidua vera by indomethacin and acetylsalicylic acid

| Addition to incubation mixtures | PGE <sub>2</sub> formed<br>(nmoles × hr <sup>-1</sup> × gm <sup>-1</sup> wet tissue) |               |              |
|---------------------------------|--------------------------------------------------------------------------------------|---------------|--------------|
|                                 | Amnion                                                                               | Chorion laeve | Decidua vera |
| No addition                     | 1.24                                                                                 | 0.29          | 0.07         |
| Indomethacin                    | 0.48                                                                                 | 0.05          | 0.01         |
| Acetylsalicylic acid            | 0.15                                                                                 | 0.03          | 0.01         |

Prostaglandin synthetase activity in homogenates of each tissue (0.5 gm wet weight) was assayed as described in "Material and methods" in the absence or presence of either indomethacin or acetylsalicylic acid. Final concentrations of indomethacin and acetylsalicylic acid were respectively 1.7 μM and 8.3 mM. After incubation for 15 minutes (for amnion) or 10 minutes (for chorion laeve and decidua vera), the prostaglandins were isolated and analyzed as described in "Material and methods." The tissues used in the experiment for which the data are shown were obtained at vaginal delivery after spontaneous onset of labor.

**Table II.** Effect of nonradiolabeled oleic acid and arachidonic acid on [<sup>14</sup>C]PGE<sub>2</sub> formation in homogenates of human amnion tissue

| Addition to incubation mixtures | PGE <sub>2</sub> formed<br>(nmoles × hr <sup>-1</sup> × gm <sup>-1</sup> wet tissue) |
|---------------------------------|--------------------------------------------------------------------------------------|
| No addition                     | 1.34                                                                                 |
| Oleic acid                      | 1.58                                                                                 |
| Arachidonic acid                | 0.07                                                                                 |

Human amnion (0.5 gm wet weight), obtained after spontaneous labor and vaginal delivery, was homogenized and incubated with 5 nmoles of [<sup>14</sup>C]arachidonic acid for 15 minutes in the absence or presence of 500 nmoles of nonradiolabeled oleic acid or arachidonic acid. After a 15-minute incubation, the prostaglandins were isolated and analyzed as described in "Material and methods."

chloroform layer was evaporated under nitrogen. The residue was dissolved in chloroform and applied to a Unisil column. The columns were washed with chloroform to remove most of the unmetabolized arachidonic acid. The prostaglandins were eluted with chloroform containing methanol (5%, v:v).<sup>19</sup> The eluate was evaporated and the residue was redissolved in a small volume of chloroform:methanol (2:1, v:v). Prostaglandins were separated by thin-layer chromatography on silica gel 60-coated plates (E. Merck, Darmstadt, Germany). The solvent systems employed for thin-layer chromatography were: system A, chloroform:methanol:acetic acid:water, 90:8:1:0.8<sup>20</sup>; system B, chloroform:methanol:acetic acid, 98:2:5.<sup>21</sup> Chromatography solvents were prepared fresh daily; chromatography tanks were lined with fil-

**Table III.** Prostaglandin production in tissue obtained after spontaneous labor and vaginal delivery and in tissues obtained at cesarean section before the onset of labor

| Tissue                                           | Prostaglandins formed<br>(nmoles $\times$ hr <sup>-1</sup> $\times$ gm <sup>-1</sup> wet tissue)* |                                     |                  |
|--------------------------------------------------|---------------------------------------------------------------------------------------------------|-------------------------------------|------------------|
|                                                  | PGE <sub>2</sub>                                                                                  | PGF <sub>2<math>\alpha</math></sub> | Peak III         |
| <i>Vaginal delivery:</i>                         |                                                                                                   |                                     |                  |
| Amnion (12)                                      | 1.43 $\pm$ 0.30                                                                                   | 0.04 $\pm$ 0.01                     | 0.18 $\pm$ 0.03† |
| Chorion laeve (12)                               | 0.48 $\pm$ 0.12                                                                                   | 0.05 $\pm$ 0.02                     | 0.27 $\pm$ 0.08† |
| Decidua vera (11)                                | 0.22 $\pm$ 0.06                                                                                   | 0.17 $\pm$ 0.04                     | 0.31 $\pm$ 0.05† |
| <i>Cesarean section (before onset of labor):</i> |                                                                                                   |                                     |                  |
| Amnion (8)                                       | 0.59 $\pm$ 0.07                                                                                   | 0.02 $\pm$ 0.00                     | 0.07 $\pm$ 0.02† |
| Chorion laeve (8)                                | 0.30 $\pm$ 0.10                                                                                   | 0.03 $\pm$ 0.01                     | 0.19 $\pm$ 0.07† |
| Decidua vera (8)                                 | 0.16 $\pm$ 0.04                                                                                   | 0.12 $\pm$ 0.01                     | 0.23 $\pm$ 0.06† |

Comparison of means was performed by means of Student's *t* test as described by Blalock.<sup>26</sup> Only the rate of PGE<sub>2</sub> formation in amnion was significantly greater (*p* < 0.02) in samples obtained after labor and normal delivery than in those obtained at cesarean section before the onset of labor. The numbers in parentheses are the number of tissue samples studied.

\*Each value presented is the mean  $\pm$  SEM.

†These values are not corrected for recovery.

ter paper and equilibrated before use. The areas on the chromatograms containing radioactive substances were localized by use of a Berthold thin-layer radiochromatogram scanner. Authentic samples of non-radiolabeled PGE<sub>2</sub>, PGF<sub>2 $\alpha$</sub> , PGB<sub>2</sub>, 15-keto-PGE<sub>2</sub>, and 15-keto-PGF<sub>2 $\alpha$</sub>  (provided by Dr. John Pike of The Upjohn Co., Kalamazoo, Michigan) were chromatographed in lanes parallel to those of the assay samples, and these compounds were localized on the chromatograms after exposure to iodine vapor. The areas of the chromatograms of the assay samples corresponding to the R<sub>f</sub> values of the known standards were scraped from the plate, dissolved in a solution of toluene containing methanol (10%, v:v) and 5-diphenylazole (0.5%, w:v), and assayed for radioactivity in a liquid-scintillation spectrometer.

All experimental values were corrected by subtracting blank values which were computed from the amount of radioactivity incorporated into products at zero incubation time. The recovery of prostaglandin products was estimated from the recovery of [<sup>3</sup>H]PGE<sub>2</sub> or [<sup>3</sup>H]PGF<sub>2 $\alpha$</sub>  from reaction mixtures that contained tissue homogenates but no substrate at zero incubation time. Recoveries for [<sup>3</sup>H]PGE<sub>2</sub> and [<sup>3</sup>H]PGF<sub>2 $\alpha$</sub>  respectively were 74% and 64%.

Under the assay conditions employed, the rate of prostaglandin formation by amnion tissue homogenates was linear up to 20 minutes at 37° C, whereas the rates of prostaglandin formation by chorion laeve and decidua vera tissue homogenates were linear up to 10 minutes at 37° C. In most experiments, homogenates prepared from 0.5 gm wet weight of each tissue were used for the measurement of prostaglandin synthetic activity. When 1.0 gm wet weight of amnion tissue was

used, the rate of PGE<sub>2</sub> formation was twice that found when 0.5 gm of tissue was used. All assays were performed either in duplicate or in triplicate.

**Assay of PGDH activity.** For the assay of PGDH activity in amnion, chorion laeve, or decidua vera tissues, 2.0 gm wet weight of each tissue was homogenized in 10 ml of either potassium phosphate buffer (50 mM, pH 7.4) containing EDTA (2 mM), or Tris-HCl buffer (50 mM, pH 7.2) containing glycerol (20%, v:v), sucrose (0.25M), dithiothreitol (2 mM), and EDTA (1 mM). The supernatant fractions, prepared by centrifugation of the tissue homogenates at 105,000  $\times$  g for 60 minutes at 2° C, were used for the assay of PGDH activity by the spectrofluorometric method described by Casey and Johnston.<sup>22</sup> Protein was measured by the method of Lowry and associates<sup>23</sup> after precipitation by trichloroacetic acid.<sup>22</sup>

## Results

**Prostaglandin synthesis and metabolism in amnion tissue.** In incubation mixtures containing amnion homogenates and [<sup>14</sup>C]arachidonic acid, a substance(s) containing considerable radioactivity was formed which comigrated with PGE<sub>2</sub> (peak II, Fig. 1) on thin-layer chromatograms. No detectable radiolabeled prostaglandins were formed when heat-denatured homogenates of amnion tissue were employed. One aliquot of the isolated radiolabeled prostaglandin product, presumed to be PGE<sub>2</sub>, was treated with alkali and another aliquot was treated by NaBH<sub>4</sub> reduction<sup>24</sup>; such treatments resulted in nearly complete conversion of the radiolabeled product to PGB<sub>2</sub> and to PGF<sub>2</sub>, respectively, as judged by thin-layer chromatography of the products with solvent system A.



These findings are supportive of the conclusion that the primary radiolabeled prostaglandin produced from [<sup>14</sup>C]arachidonic acid in incubations with amnion tissue homogenates was PGE<sub>2</sub>. In addition, a small amount of radioactive product(s) (peak III, Fig. 1) was detected which cochromatographed with 15-keto-PGE<sub>2</sub>. In incubations of amnion tissue homogenate in which NAD<sup>+</sup> (3 mM) was added (data not shown), there was no conversion of PGE<sub>2</sub> to 15-keto-PGE<sub>2</sub>. This finding is suggestive that PGDH is not present in the amnion. Thus, the identity of the radioactive product(s) in peak III as 15-keto-PGE<sub>2</sub> is questionable.

**Prostaglandin synthesis and metabolism in chorion laeve and decidua vera tissues.** With [<sup>14</sup>C]arachidonic acid used as substrate, prostaglandin formation was evaluated in incubations of homogenates of chorion laeve and decidua vera tissues in the absence or presence of NAD<sup>+</sup> (3 mM). The radiolabeled prostaglandin products were isolated as described above. In incubations of homogenates of chorion laeve in the absence of NAD<sup>+</sup>, a radiolabeled product containing considerable radioactivity and having chromatographic mobility similar to that of PGE<sub>2</sub> (peak II, Fig. 1) was formed, as was an additional radiolabeled product(s) (peak III, Fig. 1) which cochromatographed with 15-keto-PGE<sub>2</sub>.

In incubations of homogenates of decidua vera tissue in which NAD<sup>+</sup> was not added, PGE<sub>2</sub> (peak II, Fig. 1) and 15-keto-PGE<sub>2</sub> (peak III, Fig. 1) were formed as was an additional product (peak I, Fig. 1) which cochromatographed with PGF<sub>2α</sub>.

When NAD<sup>+</sup> (a cofactor for PGDH) was added to reaction mixtures containing homogenates of chorion laeve tissue, the amount of radioactivity in PGE<sub>2</sub> was markedly diminished compared to that found in incubations in which NAD<sup>+</sup> was not added. When NAD<sup>+</sup> was added to reaction mixtures containing homogenates of decidua vera tissue, the amounts of radioactivity in PGE<sub>2</sub> and in PGF<sub>2α</sub> were diminished. However, the amount of radioactivity converted to less polar metabolites (peak III and other minor peaks, Fig. 1) was increased when NAD<sup>+</sup> was added. When solvent system A was used, 15-keto-PGE<sub>2</sub> comigrated with the substance(s) occupying the area of peak III; however, it has been reported that the Rf value for 13, 14-dihydro-15-keto-PGE<sub>2</sub> is similar to that of the substance(s) in peak III.<sup>25</sup> When solvent system B was used, 15-keto-PGE<sub>2</sub> and 13, 14-dihydro-15-keto-PGE<sub>2</sub> did not comigrate. In this chromatographic system the substance(s) in peak III formed by chorion laeve tissue homogenates in the presence of NAD<sup>+</sup> comigrated with 15-keto-PGE<sub>2</sub> (data not shown). Thus, the metabolite of PGE<sub>2</sub> in chorion laeve tissue incubated with NAD<sup>+</sup> was identified as 15-keto-PGE<sub>2</sub>. However, when

**Table IV.** Specific activity of 15-hydroxyprostaglandin dehydrogenase in the cytosolic fractions of human amnion, chorion laeve, and decidua vera

| Tissue        | Specific activity of<br>15-hydroxyprostaglandin dehydrogenase*<br>(nmoles × min <sup>-1</sup> × mg <sup>-1</sup> protein) |                             |
|---------------|---------------------------------------------------------------------------------------------------------------------------|-----------------------------|
|               | Phosphate buffer†                                                                                                         | Glycerol-containing buffer† |
| Amnion        | 0.1 ± 0.06 (4)                                                                                                            | Undetectable (4)            |
| Chorion laeve | 4.0 ± 0.54 (4)                                                                                                            | 44.6 ± 11.65 (11)           |
| Decidua vera  | 1.0 ± 0.16 (3)                                                                                                            | 60.0 ± 11.58 (11)           |

\*Each value presented is the mean ± SEM. Numbers in parentheses are the numbers of tissue samples studied.

†Results obtained when tissues were homogenized in phosphate buffer or glycerol-containing buffer.

homogenates of decidua vera tissue incubated with NAD<sup>+</sup> were used, the metabolite of PGE<sub>2</sub> did not comigrate with 15-keto-PGE<sub>2</sub> when solvent system B (data not shown) was used, but this substance had an Rf value similar to that reported for 13, 14-dihydro-15-keto-PGE<sub>2</sub>.<sup>21</sup> The metabolites of PGF<sub>2α</sub> (the substances comprising the two small peaks between PGE<sub>2</sub> and 15-keto-PGE<sub>2</sub>, Fig. 1) had chromatographic mobilities corresponding to those reported for 15-keto-PGF<sub>2α</sub> and 13, 14-dihydro-15-keto-PGF<sub>2α</sub>.<sup>25</sup>

**Inhibition of prostaglandin synthetase activity.** When indomethacin or acetylsalicylic acid (compounds which inhibit cyclooxygenase and diminish prostaglandin formation) were present in the incubation medium, the rate of conversion of radiolabeled arachidonic acid to prostaglandin(s) was decreased significantly. Data for inhibition of PGE<sub>2</sub> formation are given in Table I.

The amount of radiolabeled PGE<sub>2</sub> produced by amnion tissue homogenates was also diminished significantly when nonradiolabeled arachidonic acid, in 100-fold excess, was added to the reaction mixture (Table II), whereas nonradiolabeled oleic acid (100-fold excess) did not cause a decrease in the rate of conversion of [<sup>14</sup>C]arachidonic acid to [<sup>14</sup>C]PGE<sub>2</sub>.

**Prostaglandin synthetase activity in tissues obtained prior to labor and in tissues obtained following labor.** The rates of prostaglandin formation from [<sup>14</sup>C]arachidonic acid in homogenates of tissue samples obtained after spontaneous labor and vaginal delivery and in homogenates of tissue samples obtained at cesarean section before the onset of labor were compared (Table III). When the total prostaglandin synthetase specific activity was expressed as the sum of PGE<sub>2</sub> and PGF<sub>2α</sub> production per hour per gram of wet tissue, the mean specific activity of prostaglandin synthetase in amnion was greater than that in chorion laeve tissue,

which, in turn, was greater than that in decidua vera tissue. However, by paired *t* test analysis of the data we could not establish that these relationships were statistically significant, with the exception that the total prostaglandin synthetase specific activity was greater in amnion tissue than in decidua vera tissue ( $p < 0.02$ ). This is true both in tissues obtained at cesarean section before the onset of labor and in tissues obtained at vaginal delivery following the spontaneous onset of labor. In each tissue examined, the mean total prostaglandin synthetase specific activity was higher in samples which were obtained following the spontaneous onset of labor and vaginal delivery compared to that in samples obtained at cesarean section prior to the onset of labor; however, only in the amnion tissues were there statistically significant differences between the specific activities of prostaglandin synthetase in samples obtained before and after the onset of labor ( $p < 0.02$ ). As noted previously, decidua vera tissue formed  $\text{PGF}_{2\alpha}$  at a rate approximately equal to  $\text{PGE}_2$  formation, whereas chorion laeve and amnion tissues did not form detectable amounts of radiolabeled  $\text{PGF}_{2\alpha}$  from [ $^{14}\text{C}$ ]arachidonic acid.

#### 15-Hydroxyprostaglandin dehydrogenase activity in amnion, chorion laeve, and decidua vera tissues.

To evaluate further the role of PGDH in fetal membranes and decidua vera in the metabolism of prostaglandins, the activity of this enzyme was assayed in the supernatant fractions of these three tissues by means of a spectrofluorometric assay.<sup>22</sup> We measured PGDH activity in  $105,000 \times g$  supernatant fractions which were prepared following homogenization of these tissues in either phosphate buffer (which was used for the assay of prostaglandin synthetase activity) or in a buffered medium which contained Tris, glycerol, sucrose, dithiothreitol, and EDTA to facilitate the stabilization of the enzyme.<sup>27</sup> With  $\text{PGE}_2$  used as the substrate in this assay, we again found that amnion tissue had low PGDH activity, irrespective of the homogenization medium employed (Table IV). Chorion laeve and decidua vera tissues contained PGDH and the mean specific activities of this enzyme in these two tissues were similar. Much higher PGDH specific activities were found when the tissues were prepared in the medium containing glycerol.

#### Comment

The human fetal membranes and uterine decidua are believed to play a central role in the metabolic events that lead to the initiation of parturition.<sup>1</sup> Indeed, many factors that adversely affect these tissues cause premature labor. Hypoxia, infection, or premature rupture of the fetal membranes, as well as expo-

sure of these tissues to hypertonic solutions of NaCl, glucose, or urea, cause abortion or premature labor.<sup>1</sup> Based on these observations, as well as others which are suggestive that prostaglandins are involved in the initiation of labor, we sought to characterize the biosynthesis and inactivation of prostaglandins in these tissues.

In the present investigation we found that radiolabeled arachidonic acid was converted to radiolabeled  $\text{PGE}_2$  but not to  $\text{PGF}_{2\alpha}$  in homogenates of amnion tissue. Mitchell and associates,<sup>15</sup> using a superfusion system for the measurement of the rate of release of prostaglandins and their metabolites by amnion tissue, also found that  $\text{PGE}_2$ , quantified by radioimmunoassay, was the major class of prostaglandin in the superfusate; however, they also were able to detect the release of prostacyclin<sup>28</sup> as well as small amounts of  $\text{PGF}$  and thromboxane  $\text{B}_2$ <sup>29</sup> in the superfusates of amnion tissues.

In incubations of tissue homogenates with [ $^{14}\text{C}$ ]arachidonic acid, we found that the activity of prostaglandin synthetase per gram of tissue was greater in amnion than in chorion laeve or decidua vera. We were able to establish the statistical significance of these differences only in that the activity in amnion was greater than the activity in decidua vera, a finding which is similar to that reported by Kinoshita and associates.<sup>16</sup> Mitchell and associates,<sup>15</sup> again using the superfusion system, also found greater prostaglandin release from amnion than from chorion laeve or decidua vera tissues. However, in an earlier study with the use of homogenates of amnion tissue,<sup>17</sup> this same group of investigators found no significant biosynthesis of prostaglandins or related substances.

In the present study we found that the specific activity of prostaglandin synthetase in amnion tissue obtained following the spontaneous onset of labor and vaginal delivery was significantly greater than that found in amnion tissue obtained at the time of cesarean section before the onset of labor. Mitchell and associates could not establish a statistically significant difference in the rate of release of  $\text{PGE}_2$ <sup>15</sup> and thromboxane  $\text{B}_2$ <sup>29</sup> into superfusates of amnion tissues obtained after labor compared to the rate of release from amnion tissues obtained prior to labor. However, they did find a significantly greater release of prostacyclin during the superfusion of amnion tissues obtained after vaginal delivery than from tissues obtained before labor commenced.<sup>28</sup>

We found that PGDH activity in amnion tissue was very low. In incubations of homogenates of amnion tissue with radiolabeled arachidonic acid and  $\text{NAD}^+$ , little or no radiolabeled 15-keto- $\text{PGE}_2$  was formed.



Similar results were obtained previously by others<sup>17</sup> who incubated homogenates of amnion tissue with [<sup>3</sup>H]PGF<sub>2α</sub> and NAD<sup>+</sup>. Moreover, when we used a direct assay for PGDH activity in cytosolic fractions prepared from amnion tissues, we detected very little PGDH activity. Based on these findings we conclude that there is a considerable capacity for prostaglandin synthesis in amnion and that the PGE<sub>2</sub> produced in amnion is not degraded in this tissue since PGDH, the enzyme that catalyzes the first reaction in the biodegradation of prostaglandins, is not present in significant amounts in amnion.

When we used homogenates of chorion laeve tissue, we found that radiolabeled arachidonic acid was converted to radiolabeled PGE<sub>2</sub> and 15-keto-PGE<sub>2</sub>, whereas PGF<sub>2α</sub> formation in this tissue was negligible. Other investigators<sup>16, 17</sup> also found that only PGE<sub>2</sub> was synthesized in homogenates of chorion laeve incubated in the presence of arachidonic acid. With the use of a superfusion system in which only endogenous fatty acids for prostaglandin precursors were available, it was found that both PGE and PGF were released from chorion laeve tissue into the superfusate.<sup>15</sup> The precise reason(s) why PGF release occurs during the superfusion of chorion laeve tissue is not altogether clear. However, contamination of the chorion laeve with decidua vera in such preparations is a possible explanation. This possibility is an important consideration since we found that PGF<sub>2α</sub> was formed from radiolabeled arachidonic acid in decidua vera tissue.

In the present investigation, we found that the mean of the specific activities of prostaglandin synthetase in chorion laeve tissues was lower than that in amnion tissues, although by paired *t* test analysis we could not establish that the differences were statistically significant. The mean of the specific activities of prostaglandin synthetase in chorion laeve was greater in tissues which were obtained after spontaneous labor than in tissues which were obtained before labor, but we could not establish that the differences were significant. PGDH activity in chorion laeve tissue was demonstrated. We measured the rate of formation of radiolabeled 15-keto-PGE<sub>2</sub> from radiolabeled arachidonic acid by homogenates of chorion laeve tissue with NAD<sup>+</sup> added to the incubation mixture. PGDH activity in the cytosolic fraction of chorion laeve tissue was also demonstrated by means of a spectrofluorometric assay with PGE<sub>2</sub> used as the substrate.

Radiolabeled PGE<sub>2</sub> and PGF<sub>2α</sub> were formed from radiolabeled arachidonic acid by homogenates of decidua vera tissue. Almost equal amounts of PGE<sub>2</sub> and PGF<sub>2α</sub> were formed in decidua vera tissue homogenates which were incubated under conditions identical

to those used for incubations of amnion and chorion laeve tissue homogenates in which primarily PGE<sub>2</sub> was biosynthesized. On the basis of these findings we suggest that the differences in the types of PG formed by these tissues are a reflection of the presence, or absence, of various enzymes in the tissue rather than addition of the various cofactors. PGDH activity was present in the cytosolic fraction of homogenates of decidua vera tissue and the specific activity of PGDH in this tissue as well as in chorion laeve tissue was higher than that in human placental tissue.<sup>22</sup> With [<sup>3</sup>H]PGF<sub>2α</sub> used as the substrate, Keirse and Turnbull<sup>30</sup> also found high PGDH activity in chorion laeve but low activity in decidua vera.

We found that the mean of the specific activities of prostaglandin synthetase in decidua vera tissues which were obtained after spontaneous labor and vaginal delivery was greater than that in tissues which were obtained by cesarean section prior to labor, a finding similar to that reported by Willman and Collins.<sup>14</sup> However, as in the case of chorion laeve tissue, we could not establish that the differences were statistically significant. The mean of the specific activities of prostaglandin synthetase in decidua vera was less than that in either chorion laeve or amnion although by paired *t* test analysis we found a significant difference only between the activities in decidua vera and amnion. In superfusion studies of decidua vera,<sup>15, 28, 29</sup> PGE, PGF, thromboxane B<sub>2</sub>, and prostacyclin were also detected in the superfusate.

Thus, under the conditions of these experiments, there appears to be a unique anatomic distribution of prostaglandin synthetase and PGDH among the fetal membranes and uterine decidua vera: (1) Amnion tissue formed PGE<sub>2</sub> exclusively but did not possess PGDH activity; (2) chorion laeve tissue formed PGE<sub>2</sub> exclusively and did possess PGDH activity; (3) decidua vera tissue formed both PGE<sub>2</sub> and PGF<sub>2α</sub> and also contained PGDH activity.

The fetal membranes occupy a special anatomic position, being contiguous with the amniotic fluid on one side and the decidua vera on the other; thus, the fetal membranes are in direct communication both with tissues of the mother and with the fetus through secretions of the fetal lung, skin, or urinary and gastrointestinal tracts. These avascular tissues are enriched in arachidonic acid, the obligate precursor of prostaglandins of the 2-series, and the arachidonic acid enrichment of the glycerophospholipids of these tissues uniquely involves the phosphatidylethanolamines. Indeed, 60% of the arachidonic acid in the glycerophospholipids of the amnion and chorion laeve is found in the phosphatidylethanolamines.<sup>11</sup> Considering the fatty acyl and alkyl

(phasmalogen) content of the phosphatidylethanolamines, it can be computed that 65% of the phosphatidylethanolamine molecules in the fetal membranes, prior to the onset of labor, have arachidonic acid in the *sn*-2 position.<sup>11</sup> We have also found that the arachidonic acid content of the phosphatidylethanolamines of the amnion decreases during labor<sup>11</sup> and that there is substrate specificity for the phospholipase A<sub>2</sub> of the chorioamnion; the phosphatidylethanolamine, and especially the *sn*-2-arachidonoyl esters of phosphatidylethanolamines, are the preferred substrates for the phospholipase A<sub>2</sub> of these tissues.<sup>12</sup> On the other hand, such substrate specificity could not be demonstrated for the phospholipase A<sub>2</sub> of decidua vera.<sup>12</sup>

These observations, together with the finding of a striking increase in arachidonic acid concentration of amniotic fluid during labor, are suggestive of a unique role for the fetal membranes in the initiation of human parturition. Presently the precise nature of this role must remain speculative. However, based upon the findings of the present study and those involving prostaglandin synthesis and metabolism in other tissues, we suggest that the role of the fetal membranes in the initiation of the biochemical processes that eventuate in parturition may be formulated as follows. First, a relatively large amount of arachidonic acid is released from glycerophospholipid stores in the amnion, a small portion of which serves as the precursor of PGE<sub>2</sub> in the fetal membranes and another portion may serve as the precursor of PGE<sub>2</sub> and PGF<sub>2α</sub> in the decidua vera. Second, PGE<sub>2</sub> produced in the amnion may facilitate the

release of arachidonic acid in either the chorion laeve or the uterine decidua vera and thereby facilitate the production of PGE<sub>2</sub> in the chorion and PGE<sub>2</sub> and PGF<sub>2α</sub> in the decidua vera. It has been shown that in other tissues PGE<sub>2</sub> acts to facilitate the release of arachidonic acid, e.g., from cholesterol esters in the adrenal cortex.<sup>31</sup> The earlier increase in the level of PGE<sub>2</sub> than of PGF<sub>2α</sub> in amniotic fluid before labor commences<sup>32</sup> and the earlier increase of PGE<sub>2</sub> metabolites than of PGF<sub>2α</sub> metabolites in maternal blood during labor<sup>33</sup> are consistent with this postulate. Indeed, the administration of vaginal suppositories containing PGE<sub>2</sub> to women in the second trimester of pregnancy may also facilitate the production of PGF<sub>2α</sub>. Lackritz and associates<sup>33</sup> demonstrated that PGF<sub>2α</sub> metabolites in plasma increased strikingly after such treatment. Thus, it can be envisioned that any stimulus which would facilitate PGE<sub>2</sub> synthesis in the fetal membranes, e.g. hypoxia, infection, exposure to hypertonic solutions, prostaglandins, arachidonic acid, or as yet unidentified stimuli, could serve to initiate the series of steps which eventuate in the formation of PGF<sub>2α</sub> in the decidua vera and/or in the myometrium. PGF<sub>2α</sub>, in turn, may act directly on the myometrium to initiate the uterine contractions of human parturition.

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# Estriol in pregnancy

## VII. Unconjugated plasma estriol in prolonged gestation

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This study was undertaken to determine the course of unconjugated plasma estriol ( $E_3$ ) concentrations after 40 weeks' gestation and to assess whether the latter may be used as a first-line test in the management of term pregnancies with poor dates and prolonged gestation. Plasma  $E_3$  was measured twice weekly in 134 women with well-dated pregnancies of 40 weeks or more and in 213 women with pregnancies of unverified age but believed to be in excess of 40 weeks from the last menstrual period. The obstetric management was based upon twice weekly determinations of urinary  $E_3$  and antepartum heart rate testing, but not all patients complied. Plasma  $E_3$  levels were not available for management of the patients. The results indicate that unconjugated plasma  $E_3$  levels are highest at 40 weeks' gestation and decline thereafter by 12% each week. Because of this slow decline, biweekly assays suffice. Increasing and plateauing serial plasma  $E_3$  levels reveal that a patient approaches term or has reached 39 to 41 weeks' gestation, respectively, whereas decreasing  $E_3$  concentrations indicate that the patient is well beyond 40 weeks, and that her fetus may be about to or has become postmature. Only seven of the 347 patients studied were delivered of a postmature infant. Their last plasma  $E_3$  levels ranged from 3.8 to 8.0 ng/ml, i.e., were below the 95% confidence limits established for normal term pregnancies. It was found that 73% of the 347 patients had plasma  $E_3$  levels of 12 ng/ml or more, and that all their pregnancies ended normally, except for three instances of presumed intrapartum fetal distress. However, in 19 (20%) of the other 27% of the 347 patients who had plasma  $E_3$  levels of less than 12 ng/ml, abnormal outcome was encountered: postmaturity (seven), fetal death (four), intrauterine growth retardation (three), malformations (two), or intrapartum fetal distress (three). These results allow the conclusion that twice weekly assays of unconjugated plasma  $E_3$  may be used as a first-line test in the management of prolonged gestation. (AM. J. OBSTET. GYNÉCOL. 139:382, 1981.)

PROLONGED GESTATION usually is defined as a pregnancy which exceeds 294 days calculated from the first day of the last menstruation in a 28-day cycle.

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Whether prolongation of gestation by itself constitutes an increased risk of perinatal morbidity and mortality has been and remains a matter of dispute.<sup>1</sup> Those obstetricians who regard postdate pregnancy as a serious threat to the fetus advocate delivery at 42 weeks' gestation.<sup>2</sup> Other authors, however, believe that the potential complications resulting from induction of labor and possible cesarean section for failed induction or non-propagation of labor, undertaken solely on account of prolonged gestation, outweigh the advantages of obstetric intervention at 42 weeks.<sup>3</sup> This problem could be resolved in a rational manner if it were possible to reliably distinguish between a healthy fetus in an otherwise uncomplicated pregnancy and a postmature (dysmature) fetus who must be delivered without delay. Assays of urinary estriol ( $E_3$ ) have been reported to allow individualization of the management of prolonged pregnancies.<sup>4, 5</sup>



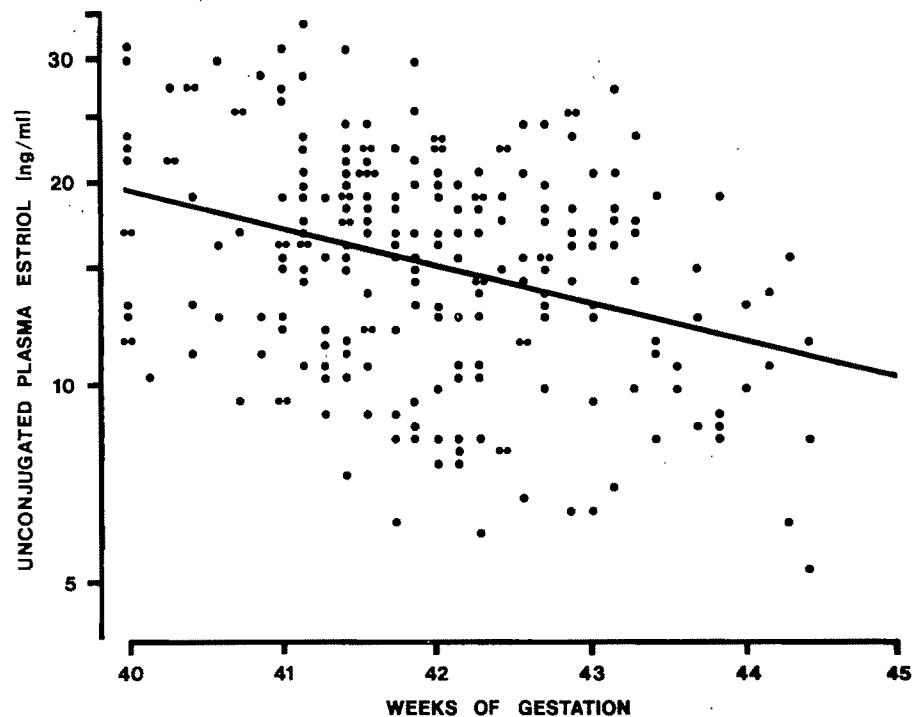


Fig. 1. Unconjugated plasma estriol concentrations in 134 women at 40 weeks' gestation and beyond. The regression line ( $y = -0.00801x + 3.53078$ ;  $x$  = days from last menstrual period,  $y = \log_{10}$  unconjugated plasma estriol level in ng/ml;  $r = 0.34099$ ,  $p < 0.00001$ ) indicates a 12.1% decrease per week after 40 weeks.

Table I. Unconjugated plasma estriol concentrations in prolonged gestation

| Duration of gestation | Mean (ng/ml) | Median (ng/ml) | SD (ng/ml) | 95% CL* (ng/ml) | n  |
|-----------------------|--------------|----------------|------------|-----------------|----|
| 40 wk-40 wk, 6 days   | 18.6         | 17.9           | ±6.8       | 8.7-36.         | 31 |
| 41 wk-41 wk, 6 days   | 17.2         | 16.6           | ±5.9       | 6.6-34.         | 85 |
| 42 wk-42 wk, 6 days   | 15.3         | 15.7           | ±5.3       | 5.8-33.         | 70 |
| 43 wk-43 wk, 6 days   | 13.7         | 13.0           | ±5.4       | 5.7-27.         | 32 |
| 44 wk-44 wk, 6 days   | 10.1         | 9.9            | ±3.4       | 4.5-21.         | 11 |

\*95% Confidence limits were determined by probit or rankit analysis.

Encouraged by our favorable experience with determinations of urinary  $E_3$  in the management of prolonged gestation,<sup>6</sup> which is in agreement with that of other investigators,<sup>4, 5, 7, 8</sup> and discouraged by the inconvenience, delay, and inaccuracy of 24-hour collections of urine, we undertook this study in an effort to determine whether measurements of unconjugated plasma  $E_3$  might be used to identify the fetus at risk in postdate pregnancies and which testing criteria must be met at all times in order to avoid fetal distress and death. The second aim of this investigation was to assess whether it might be possible to distinguish between preterm, term, and postterm pregnancies by serial assays of unconjugated plasma  $E_3$  in order to avoid untimely intervention in pregnancies with uncertain or unverifiable ("poor") dates.

Material and methods

**Patients.** Included in this study were 347 patients, 134 with well-dated and 213 with poorly dated pregnancies. Patients with preeclampsia, apparent intrauterine growth retardation (IUGR), class A diabetes mellitus,<sup>9</sup> Rh-immunization, and/or multiple pregnancies were excluded. Those patients who had a positive urinary pregnancy test by 6 weeks' gestation, a gynecologic examination before 12 weeks, and/or ultrasonography prior to 28 weeks with findings commensurate with gestational age by last menstrual period were considered to have good dates. All other patients were considered to have poor dates.

Patients with good dates were followed with weekly clinic visits, twice weekly determinations of 24-hour urinary  $E_3$  and creatinine (C), and/or once-a-week an-

**Table II.** Detailed listing of abnormal outcome which occurred in 22 of the 347 pregnancies studied

| Patient No.                                        | Plasma E <sub>3</sub> * (ng/ml) | Comments                                                                            | 5 min Apgar score | Neonate                                      |
|----------------------------------------------------|---------------------------------|-------------------------------------------------------------------------------------|-------------------|----------------------------------------------|
| <i>Postmature neonates:</i>                        |                                 |                                                                                     |                   |                                              |
| 1                                                  | 6.8                             | Fetal distress, cesarean section                                                    | 9                 | Well                                         |
| 2                                                  | 4.2                             | Precipitate delivery in parking lot outside hospital                                | ?                 | Meconium aspiration                          |
| 3                                                  | 4.2                             | Failed induction, cesarean section                                                  | 9                 | Well                                         |
| 4                                                  | 3.8                             | Induction, outlet forceps                                                           | 8                 | Well                                         |
| 5                                                  | 8.0                             | Fetal distress, cesarean section                                                    | 1                 | Seizures                                     |
| 6                                                  | 6.9                             | Spontaneous vaginal delivery                                                        | 8                 | Well                                         |
| 7                                                  | 3.9                             | Spontaneous vaginal delivery                                                        | 9                 | Well                                         |
| <i>Intrauterine fetal death (no postmaturity):</i> |                                 |                                                                                     |                   |                                              |
| 8                                                  | 5.2                             | Nonreactive NST, negative CST*†                                                     |                   | Fetal death 3 days after last E <sub>3</sub> |
| 9                                                  | 5.8                             | Unable to bring patient to hospital                                                 |                   | Fetal death 7 days after last E <sub>3</sub> |
| 10                                                 | 10.8                            | No heart rate testing                                                               |                   | Fetal death 3 days after last E <sub>3</sub> |
| 11                                                 | 11.4                            | No heart rate testing, 50% fall in unconjugated plasma E <sub>3</sub> within 1 week |                   | Fetal death 4 days after last E <sub>3</sub> |
| <i>Intrauterine growth retardation:</i>            |                                 |                                                                                     |                   |                                              |
| 12                                                 | 6.1                             | Spontaneous vaginal delivery                                                        | 10                | Well                                         |
| 13                                                 | 7.7                             | Spontaneous vaginal delivery                                                        | 9                 | Well                                         |
| 14                                                 | 5.4                             | Spontaneous vaginal delivery                                                        | 9                 | Gastroschisis                                |
| <i>Malformations:</i>                              |                                 |                                                                                     |                   |                                              |
| 15                                                 | 0.7                             | Repeat cesarean section                                                             | 5                 | Anencephalic                                 |
| 16                                                 | 10.8                            | Spontaneous vaginal delivery                                                        | 9                 | Turner's syndrome                            |
| <i>Intrapartum fetal distress:</i>                 |                                 |                                                                                     |                   |                                              |
| 17                                                 | 6.4                             | Fetal distress cesarean section                                                     | 9                 | Well                                         |
| 18                                                 | 8.4                             | Prolapsed cord, cesarean section                                                    | 8                 | Well                                         |
| 19                                                 | 9.1                             | Fetal distress cesarean section                                                     | 9                 | Well                                         |
| 20                                                 | 12.3                            | Fetal distress cesarean section                                                     | 8                 | Well                                         |
| 21                                                 | 15.2                            | Fetal distress cesarean section                                                     | 6                 | Well                                         |
| 22                                                 | 19.5                            | Fetal distress cesarean section                                                     | 6                 | Meconium aspiration                          |

\*Last unconjugated plasma estriol (E<sub>3</sub>) measured prior to delivery.†This case was presented by Evertson and associates.<sup>9</sup>

intrapartum fetal heart rate testing. Induction of labor was initiated at or after 42 weeks as soon as the cervix was found to be favorable for induction.<sup>10</sup> If the 24-hour urinary E<sub>3</sub> excretion fell below 30 mg/24 hours or antepartum fetal heart rate testing became abnormal, i.e., in the presence of a nonreactive nonstress test (NST) followed by a positive contraction stress test (CST), induction of labor was also started. If necessary, because of failed induction or nonpropagation of labor, patients underwent delivery by cesarean section.

Patients with poor dates were referred to our clinic at or after 40 weeks' gestation according to the most likely estimate of their date of confinement. These women were seen once a week in clinic and were followed with twice weekly determinations of 24-hour urinary E<sub>3</sub> and C and/or antepartum fetal heart rate testing. Normal urinary E<sub>3</sub> excretion and/or fetal heart rate precluded obstetric intervention. If the E<sub>3</sub> excretion was less than 30 mg/24 hours, antepartum fetal heart rate testing was performed. If both E<sub>3</sub> excretion and heart rate tests were abnormal, the patient underwent delivery (either vaginally after induction of labor or abdominally

if the former was unsuccessful) if intrapartum fetal distress occurred or if there were other indications for cesarean section. Not all of the patients included in this study complied with this management scheme.

Prolonged gestation was defined in this study as a pregnancy of more than 42 completed weeks of gestation or at least 22 weeks of fetal heart tones observed by auscultation. Normal outcome was defined as absence of fetal distress during labor and delivery, a 5 minute Apgar score of at least 7, normal findings on examination of the neonate (i.e., no signs of postmaturity), and an uncomplicated neonatal course. Abnormal outcome, on the other hand, included intrapartum fetal distress as evidenced by abnormal fetal heart rate patterns consisting of repetitive late decelerations and/or prolonged bradycardia (i.e., less than 90 beats per minute for more than 2 minutes), scalp pH values below 7.20,<sup>11</sup> and/or moderate or thick meconium, a 5 minute Apgar score below 7, evidence of postmaturity by pediatric examination, IUGR, and fetal death.

**Testing.** Antepartum fetal heart rate testing was performed as previously described,<sup>12, 13</sup> with use of the



**Table III.** Normal and abnormal outcome in 347 well-dated prolonged gestations or poorly dated term, postdate, or postmature pregnancies in relation to the last unconjugated plasma estriol ( $E_3$ ) level measured within 7 days prior to delivery

| Outcome  | Unconjugated plasma $E_3$ |                 | All patients |
|----------|---------------------------|-----------------|--------------|
|          | <12 ng/ml                 | $\geq$ 12 ng/ml |              |
| Normal   | 76                        | 249             | 325          |
| Abnormal | 19                        | 3               | 22           |
| Total    | 95 (27%)                  | 252 (73%)       | 347 (100%)   |

Figures indicate number of patients.  
 $p < 0.005$ , chi-square analysis.

**Table IV.** Normal and abnormal outcome in 95 pregnant women who had either well-dated prolonged gestations or poorly dated term, postdate, or postmature pregnancies and whose last unconjugated plasma estriol ( $E_3$ ) measured within 7 days prior to delivery was below 12 ng/ml in relation to the last plasma  $E_3$  concentration

| Outcome  | Unconjugated plasma $E_3$ |                | All patients |
|----------|---------------------------|----------------|--------------|
|          | <7 ng/ml                  | $\geq$ 7 ng/ml |              |
| Normal   | 7                         | 69             | 76           |
| Abnormal | 11                        | 8              | 19           |
| Total    | 18                        | 77             | 95           |

$p < 0.005$ , chi-square analysis.

NST<sup>14</sup> as the initial test, and, if that was nonreactive, the CST.<sup>15</sup> Urinary  $E_3$  was measured as previously reported from this laboratory.<sup>16</sup> Creatinine was measured simultaneously to correct for inadequate collection of urine. If a specimen of urine contained less than 1.0 gm of C, the amount of  $E_3$  determined in this specimen was multiplied by 1.3, the average 24-hour excretion of C in late pregnancy,<sup>17</sup> and divided by the amount of C found in the specimen. If a specimen of urine contained less than 0.5 gm of C, the  $E_3$  results were disregarded because the inaccuracy of such a collection of urine might make the result inaccurate even if it has been corrected for C excretion.<sup>6</sup>

Unconjugated ("free") plasma  $E_3$  was measured as previously described.<sup>18</sup> Plasma  $E_3$  results were not available for management of the patients. Included in this study were only those patients in whom the last measurement of plasma  $E_3$  was made within 7 days prior to delivery or fetal death.

**Results**

**Unconjugated plasma  $E_3$  in uncomplicated pregnancies at and after 40 weeks' gestation.** The mean

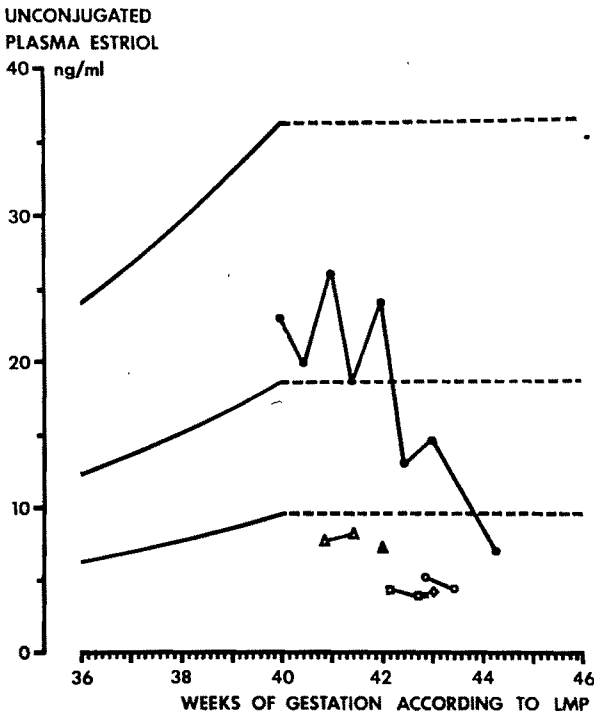


**Fig. 2.** Unconjugated plasma estriol concentrations in seven patients who were delivered of postmature infants as determined by pediatric examination. The symbols refer to patient Nos. 1 through 7 listed in Table II:  $\bullet$  (1),  $\circ$  (2),  $\diamond$  (3),  $\blacksquare$  (4),  $\triangle$  (5),  $\blacktriangle$  (6),  $\square$  (7).

unconjugated plasma  $E_3$  levels and their 95% confidence limits measured in the 134 pregnant women with good dates are listed in Table I. The average plasma  $E_3$  concentration determined in this study for 40 weeks' gestation was essentially the same as that found in a previous study for uncomplicated third trimester pregnancies.<sup>19</sup> As found in the latter study for late pregnancy, unconjugated plasma  $E_3$  levels after 40 weeks' gestation were log-normally distributed. When the logarithms of the individual plasma  $E_3$  levels were plotted versus weeks of gestation, a progressive decline in unconjugated plasma  $E_3$  was found ( $p < 0.001$ ). Regression analysis revealed that this decline averaged 12% per week for each week after 40 weeks' gestation (Fig. 1).

**Pregnancy outcome and last plasma  $E_3$  levels measured within 7 days of delivery.** As listed in detail in Table II, outcome was abnormal in 22 of the 347 pregnancies studied. In 19 of these 22 patients, the last plasma  $E_3$  measured within 7 days prior to delivery was less than 12 ng/ml. Abnormal outcome in the other three patients with plasma  $E_3$  levels above 12 ng/ml (range, 12 to 19 ng/ml) consisted of apparent intrapartum fetal distress, which was readily recognized by fetal monitoring. All three patients were delivered

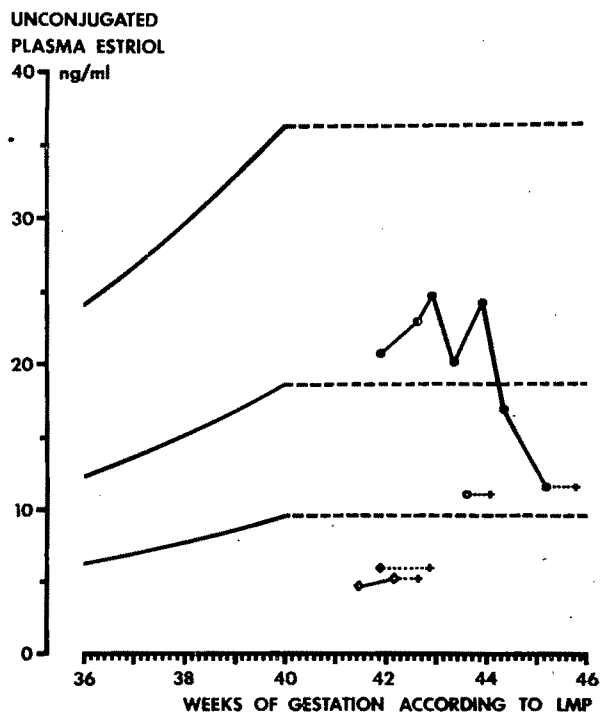


Fig. 3. Unconjugated plasma estriol concentrations in four patients whose pregnancy ended in fetal death on the day indicated by a cross. The symbols refer to patients Nos. 8 through 11 listed in Table II:  $\diamond$  (8),  $\diamond$  (9),  $\circ$  (10),  $\bullet$  (11).

of normal infants who had 5 minute Apgar scores of 7 or above.

Among the 347 patients studied, 252 (73%) had plasma  $E_3$  levels of 12 ng/ml or more, whereas in 95 women (27%) the plasma  $E_3$  concentration measured within 1 week prior to delivery was less than 12 ng/ml (Table III). Outcome in 19 of these 95 pregnancies (20%) with plasma  $E_3$  levels below 12 ng/ml was abnormal, whereas only three of the 252 (1.2%) patients with plasma  $E_3$  of at least 12 ng/ml experienced intrapartum fetal distress. This difference is significant ( $p < 0.005$ ).

Subdivision of the 95 pregnancies with plasma  $E_3$  concentrations of less than 12 ng/ml into 18 with  $E_3$  below 7 ng/ml and 77 with  $E_3$  of 7 ng/ml and above reveals that abnormal outcome is much more likely to occur if the plasma  $E_3$  level is less than 7 ng/ml ( $p < 0.005$ ), as shown in Table IV.

As outlined in Table II, only seven of the 347 patients (two with good dates and five with poor dates) were delivered of postmature infants. Three patients required cesarean section: one for failed induction, and two for intrapartum fetal distress. Plasma  $E_3$  in these patients ranged from 3.8 to 8.0 ng/ml, averaging 5.4 ng/ml (Fig. 2).

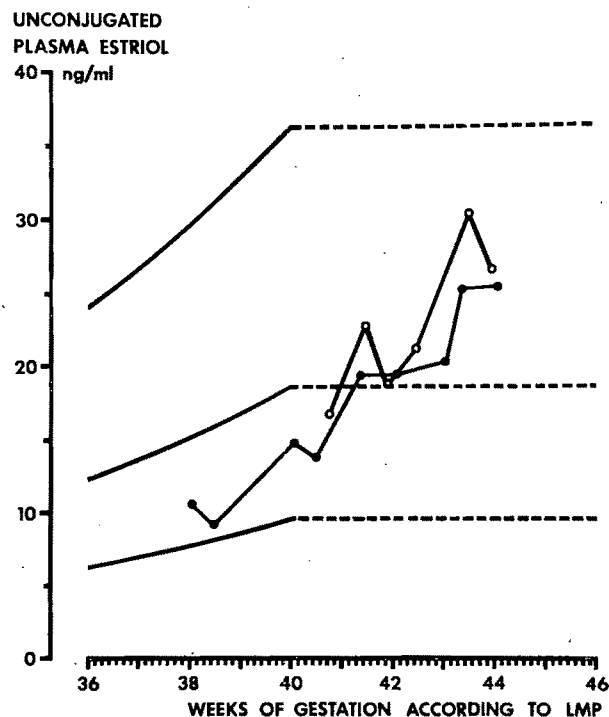


Fig. 4. Serial determinations of unconjugated plasma estriol in two patients with unverified dates. Both patterns are consistent with the pronounced rise in estriol which occurs between 36 and 40 weeks. Both patients were obviously at term when, on the basis of their last menstrual period, they were supposedly 44 weeks' pregnant. Both were delivered vaginally of healthy term infants after spontaneous onset of labor.

Fetal death occurred in four of the 347 pregnancies (11.5/1,000). It is conceivable that fetal death could have been avoided had the patients not neglected to return for weekly clinic visits and twice weekly assays of urinary  $E_3$  as well as appropriate antepartum fetal heart rate testing (Table II). The plasma  $E_3$  levels observed in these four pregnancies with fetal death are depicted in Fig. 3. The final  $E_3$  level of 11.4 ng/ml in Patient No. 11 (Table II) 6 days prior to documentation of fetal death represents a 50% drop from the previous plateau of 22 ng/ml, thus indicating that fetal distress and death were heralded by a well-advanced progressive decline in plasma  $E_3$ .

Three pregnancies with plasma  $E_3$  ranging from 5.4 to 7.7 ng/ml were complicated by unrecognized IUGR. One of the three growth-retarded neonates had gastroschisis. Two newborn infants with plasma  $E_3$  levels below 12 ng/ml had malformations: one had Turner's syndrome, and one had anencephaly (Table II).

Six pregnancies were complicated by intrapartum fetal distress only. In three of these six, the plasma  $E_3$



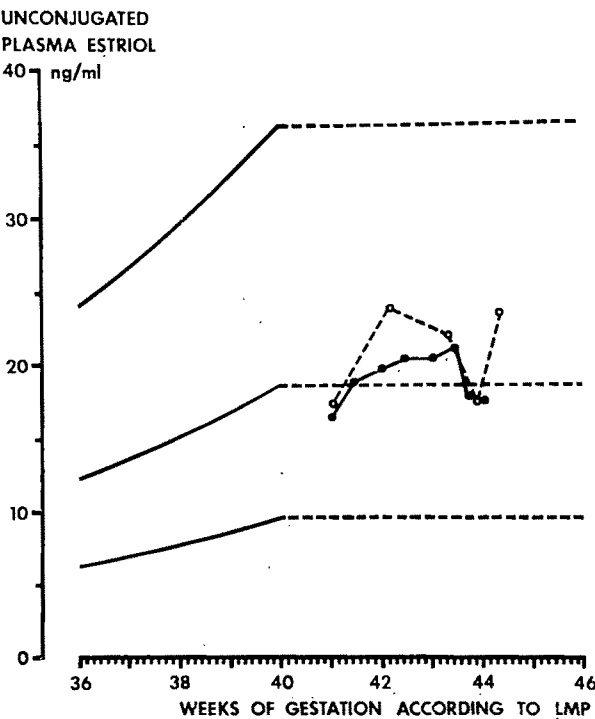


Fig. 5. Serial determinations of unconjugated plasma estriol in two patients with unverified dates who on the basis of their last menstrual period were believed to be postterm. The plateauing of the plasma estriol levels is consistent with term pregnancy. The absolute estriol levels are consistent with fetal well-being. Both patients were delivered vaginally of healthy term infants after spontaneous onset of labor.

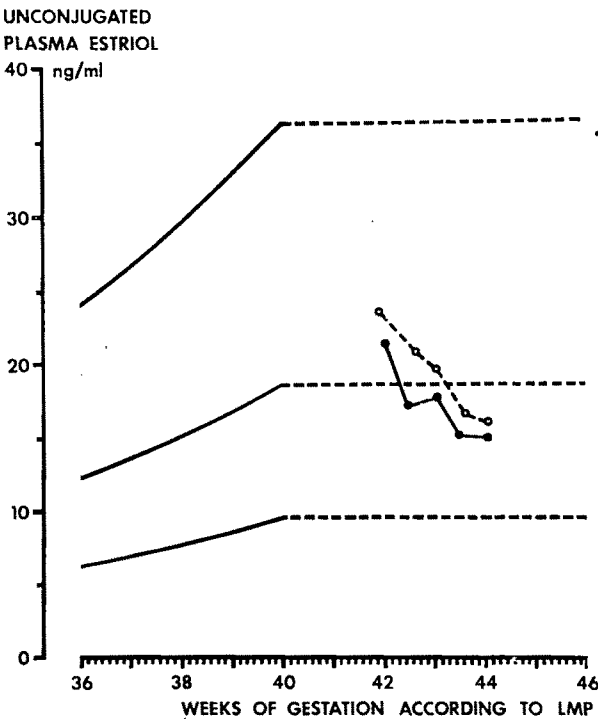


Fig. 6. Serial determinations of unconjugated plasma estriol in two patients with unverified dates. The slow but progressive decline in unconjugated plasma estriol levels is consistent with postdate pregnancy and thus commensurate with the gestational age calculated on the basis of their last menstrual period. Both patients underwent spontaneous onset of labor and were delivered vaginally of healthy infants without apparent postmaturity.

levels were 12.3, 15.2, and 19.5 ng/ml; and in the other three, the plasma  $E_3$  concentrations were below 12 ng/ml (Table II).

**Plasma  $E_3$  patterns which rule out prolonged gestation and postmaturity.** Figs. 4, 5, and 6 depict plasma  $E_3$  levels in patients with inaccurate dates who by menstrual history were believed to be postdates, but who on account of their plasma  $E_3$  patterns could be considered as approaching 40 weeks, as being at term, or as being postterm, respectively, on the basis of progressively rising, plateauing, or decreasing plasma  $E_3$  values. The pregnancies of all patients whose plasma  $E_3$  levels are depicted in Figs. 4, 5, and 6 had a good outcome of spontaneous vaginal delivery.

#### Comment

It is well established that unconjugated plasma (or serum)  $E_3$  concentrations rise more rapidly during the last month of pregnancy,<sup>19</sup> paralleling the accelerated rise in urinary  $E_3$  excretion.<sup>21</sup> In most normal pregnancies, the rate at which circulating  $E_3$  levels rise in-

creases sharply at 36 weeks.<sup>22</sup> The data obtained in this study demonstrate that the peak plasma  $E_3$  concentration reached at term is followed by a slow but progressive decline that averages 12% per week. This fall in unconjugated plasma  $E_3$  parallels the decline in total plasma  $E_3$ <sup>23</sup> as well as urinary  $E_3$ ,<sup>5</sup> and, in all likelihood, is due to placental senescence<sup>1</sup> that results in decreasing fetoplacental production of  $E_3$ . This postterm decline in  $E_3$  levels proceeds so slowly that twice weekly assays of  $E_3$  suffice for safe management of the patient. We prefer determinations of unconjugated plasma  $E_3$  because blood is more readily and conveniently obtained than is the 24-hour sample of urine, and unconjugated plasma  $E_3$  is most easily measured.<sup>6</sup> In a recent study on 70 pregnant diabetic women, we found that day-to-day and minute-to-minute fluctuations of unconjugated plasma  $E_3$  do not interfere with the successful clinical use of unconjugated plasma  $E_3$  assays.<sup>13</sup> The data obtained in this study have all been derived from single specimens of blood drawn between 8 and 11 A.M.

The typical pattern of serial levels of unconjugated

plasma  $E_3$  established for preterm, term, and postterm pregnancies consists of a rise until term, a plateau at term, and a decline thereafter. Our experience has shown that by serial determinations of unconjugated plasma  $E_3$  it is possible, in most patients, to distinguish a gestation which is approaching term from a term or postdate pregnancy. Serial assays of  $E_3$ , particularly when begun prior to the marked rise in  $E_3$  at 36 weeks' gestation,<sup>22</sup> permit us to estimate that the probable time of delivery will be  $4 \pm 1$  weeks after the rise. Many of our patients who present with uncertain or unverified dates, because of a lack of prior prenatal care, and a history consistent with term or prolonged gestation are, in fact, barely at or several weeks prior to term. Immediate delivery in these patients would constitute unnecessary intervention and, in many instances, result in iatrogenic prematurity or unnecessary cesarean section for failed induction or nonpropagation of labor. This can be safely avoided by serial determinations of unconjugated plasma  $E_3$ .

The second aim of this investigation was to test in a blinded prospective manner whether assays of unconjugated plasma  $E_3$  could be employed to identify the fetus at risk in prolonged gestation. Our data demonstrate that low and/or falling levels of unconjugated plasma  $E_3$  allow differentiation between uncomplicated prolonged pregnancies and those which require further testing and/or early delivery. The results indicate that fetal outcome was normal in all instances in which levels of unconjugated plasma  $E_3$  were at least 12 ng/ml. It must be emphasized that this value depends upon the method used,<sup>18</sup> and that it may vary if another  $E_3$  procedure is employed. For comparison, approximately 75% of all term patients assayed by our method<sup>18</sup> have unconjugated plasma  $E_3$  levels which exceed 12 ng/ml.

All but three of the 22 patients whose pregnancy outcome was abnormal had a plasma  $E_3$  of less than 12 ng/ml. The other three patients with plasma  $E_3$  concentrations of 12.3 to 19.5 ng/ml encountered intrapartum fetal distress, a condition which is readily recognized and remedied by prompt delivery. This indicates that an apparently normal plasma  $E_3$  level does not guarantee absence of intrapartum fetal distress. The seven patients who were carrying postmature fetuses had distinctively low levels of plasma  $E_3$  (3.8 to 8.0 ng/ml), and one patient showed a progressive fall over a period of 2 to 3 weeks, thereby documenting that postmaturity is associated with low and/or progressively declining concentrations of unconjugated plasma  $E_3$ .

Four pregnancies ended in fetal death: all of these patients had plasma  $E_3$  levels of less than 12 ng/ml. Of the four patients, the one who had the highest plasma

$E_3$  (11.4 ng/ml) experienced a 50% fall in plasma  $E_3$ . It is of interest to note that one of the four patients had a nonreactive NST but a negative CST 2 to 3 days before the fetus died.<sup>20</sup> The other eight of the 19 patients with plasma  $E_3$  levels of less than 12 ng/ml had the following complications: unrecognized IUGR (three), anencephaly (one), Turner's syndrome (one), and intrapartum fetal distress (three).

It must be emphasized that only 20% ( $n = 19$ ) of the 95 patients with plasma  $E_3$  levels of less than 12 ng/ml had evidence of postmaturity, antepartum fetal distress, intrapartum fetal distress, IUGR, or malformations. The other 80% ( $n = 76$ ) had no problems, underwent spontaneous onset of labor, and were delivered of normal healthy infants. On the other hand, 73% ( $n = 252$ ) of all women tested had unconjugated plasma  $E_3$  levels of at least 12 ng/ml and did well, except for three cases of intrapartum fetal distress which were readily recognized and remedied by cesarean section. These results demonstrate that low ( $< 12$  ng/ml by this method) and/or falling concentrations of unconjugated plasma  $E_3$  identify those prolonged and complicated term gestations which may need obstetric intervention. To determine whether expedient delivery is indeed necessary, additional testing (NSTs, CSTs) is required in order to avoid unnecessary intervention in about four of five patients. Contrary to the limited predictive value of low plasma  $E_3$  levels or abnormal plasma  $E_3$  patterns, a normal plasma  $E_3$  level and/or pattern which was found in three fourths of our patients was a reliable indicator of fetal well-being. Thus, twice weekly determinations of unconjugated plasma  $E_3$  appear to be a practical first-line test in the management of prolonged gestation. Heart rate monitoring may be reserved for about one fourth of all patients tested who have an abnormal  $E_3$  result. It should be emphasized that most patients with prolonged gestation and abnormal levels of unconjugated plasma  $E_3$  do not experience postmaturity, fetal death, or intrapartum distress, requiring that potential fetal jeopardy as suggested by low and/or falling plasma  $E_3$  levels be confirmed by fetal heart rate testing. However, our data also show that the lower the level of plasma  $E_3$ , the greater is the likelihood of fetal distress and/or postmaturity.

We wish to emphasize that  $E_3$  assays must not replace sound obstetric management: postdate pregnancies with a favorable cervix<sup>10</sup> deserve induction of labor, and only patients with otherwise uncomplicated pregnancies are candidates for expectant management. Since the principal objective of the use of  $E_3$  assays in prolonged gestation is the avoidance of unnecessary intervention and possible cesarean section, obviously all



patients with a term pregnancy who are candidates for cesarean section should undergo delivery promptly, provided that the amniotic fluid lecithin/sphingomyelin ratio indicates fetal maturity. Although the data derived in this study indicate that serial assays of unconjugated plasma  $E_3$  may be used as a first-line test in prolonged gestation and term or postterm pregnancies with poor dates, further prospective studies in

which plasma  $E_3$  is used to manage these patients are necessary to conclude clinical efficacy.

We are indebted to Paula Broussard, R.N., Dorothy McCart, R.N., and Marci Hamilton, R.N., for their expert and dedicated efforts in performing antepartum fetal heart rate testing, to Miss Yvonne Livett for expert technical assistance, and to Mr. Gregory E. Gray for assistance with computerized data analysis.

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# Amniotic fluid C-peptide as an index for intrauterine fetal growth

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Amniotic fluid C-peptide (AFCP) was monitored as an indicator of the amount of insulin secreted by the fetus in utero. Levels of amniotic fluid and cord blood C-peptide, insulin, and glucose were measured in 103 nondiabetic infants at  $\geq 36$  weeks' gestation. Infants were grouped according to birth weight and gestational age at delivery, as follows: small for gestational age (SGA,  $\leq 10\%$ ,  $n = 11$ ), average for gestational age (AGA, 10% to 90%,  $n = 75$ ), large for gestational age (LGA,  $> 90\%$ ,  $n = 17$ ). AFCP correlated best with infant weight-gestational age percentile classification: low AFCP in SGA infants and high AFCP in LGA infants. The data from this study suggest that a persistently low production of insulin by SGA fetuses and a high production of insulin by LGA fetuses may lead to the different intrauterine growth rates observed. (AM. J. OBSTET. GYNECOL. 139:390, 1981.)

INTRAUTERINE growth retardation (IUGR) accounts for a substantial increase in perinatal morbidity and mortality.<sup>1-4</sup> Growth retardation is often recognized early in the third trimester and may be caused by various maternal conditions that decrease uteroplacental blood flow.<sup>5</sup> Insulin has been considered to be a key regulator of fetal growth during intrauterine life<sup>6</sup> by stimulating the cellular uptake of amino acids and the subsequent synthesis of proteins. In addition, insulin enhances the deposition of glycogen and lipids in storage tissues, such as liver, heart, muscle, and fatty tissues. It is widely accepted that the mean level of glucose in fetal blood,

which depends on the placental transfer of glucose from the maternal circulation, is the major determinant of the concentration of insulin in fetal blood.<sup>7</sup> Thus, maternal hyperglycemia in diabetic patients stimulates fetal hyperinsulinism,<sup>8</sup> which is probably the underlying mechanism of excessive fetal growth in uncontrolled diabetic patients. IUGR fetuses, on the other hand, are characterized by deprivation of storage of glycogen in liver and heart, and diminished subcutaneous fatty tissue.<sup>9</sup> Their growth rates are retarded during the phase of most rapid intrauterine growth, from approximately the thirtieth to the thirty-eighth weeks of gestation. Therefore, it is reasonable to speculate that IUGR might be associated with a persistently low production of insulin by the fetus.

C-peptide is formed when proinsulin is converted to insulin in the pancreatic beta cells. Measurement of C-peptide, which is secreted in equimolar amounts with insulin, provides an accurate means of assessing endogenous secretion of insulin despite the presence of insulin antibodies. Unlike insulin, C-peptide is minimally metabolized by the liver, and its clearance is handled mainly by the kidneys.<sup>10</sup> Therefore, the level of C-peptide in amniotic fluid may be a useful reflection of the amount of insulin secreted by the fetal

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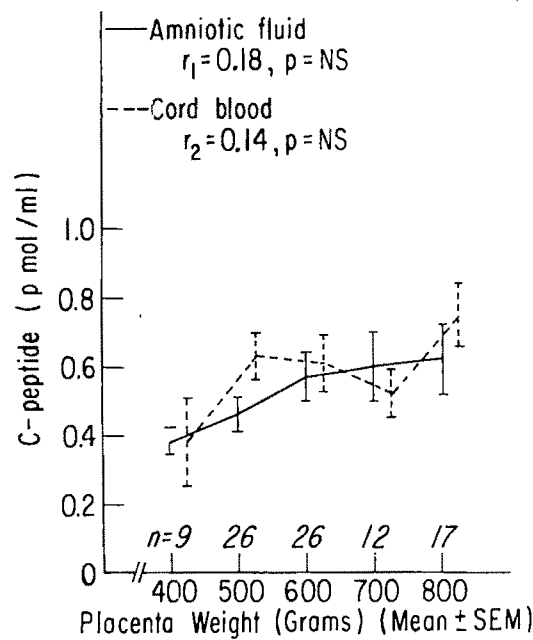


Fig. 1. The relationship between amniotic fluid and cord blood C-peptide levels and placental weight.

Table I. Comparison of C-peptide levels in maternal venous, umbilical arterial, and umbilical venous blood (n = 20)

| Source of blood  | C-peptide level (pmol/ml)<br>(Mean $\pm$ SD) |
|------------------|----------------------------------------------|
| Maternal vein    | 4.38 $\pm$ 2.25                              |
| Umbilical artery | 0.72 $\pm$ 0.32                              |
| Umbilical vein   | 0.73 $\pm$ 0.23                              |

pancreas. The availability of an accurate and sensitive method for assaying C-peptide prompted the design of this prospective controlled study.

Subjects and methods

Between June, 1979, and February, 1980, we studied the levels of amniotic fluid and cord blood C-peptide, insulin, and glucose in 103 nondiabetic infants at the Chicago Lying-In Hospital.

Patients who were suspected of IUGR, on the basis of serial measurements of fundal height or ultrasonic biparietal diameter that showed retarded growth of at least 3 weeks' lag for gestational age, were identified prenatally from our high-risk clinic population. Control patients were selected from our obstetric population in which amniocentesis was required to document fetal maturity prior to an induction of labor or a repeat cesarean section. Amniocentesis was performed at 36 weeks' gestation or beyond according to clinical or ul-

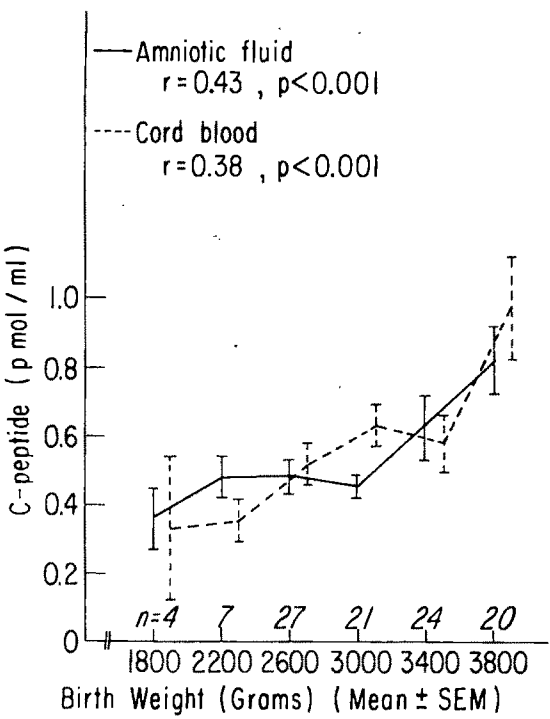


Fig. 2. The relationship between amniotic fluid and cord blood C-peptide levels and infant birth weight.

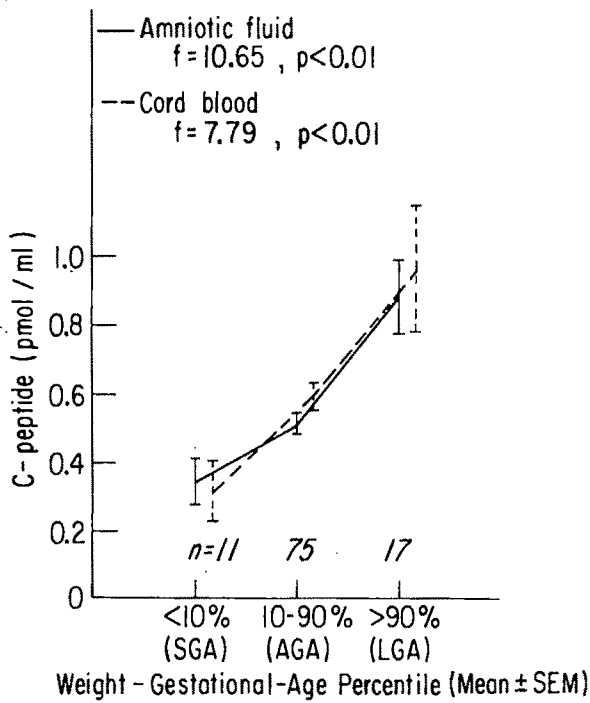


Fig. 3. The relationship between amniotic fluid and cord blood C-peptide levels and infant weight-gestational age percentile classification. Statistical comparison by one-way analysis of variance.

**Table II.** Correlation of amniotic fluid and cord blood C-peptide, insulin, and glucose levels (mean ± SEM) with infant birth weight

| Birth weight groups (gm) | Case No. (n) | Amniotic fluid        |                  |                     | Cord blood            |                  |                     |
|--------------------------|--------------|-----------------------|------------------|---------------------|-----------------------|------------------|---------------------|
|                          |              | C-peptide (pmoles/ml) | Insulin (μU/ml)* | Glucose (mg/100 ml) | C-peptide (pmoles/ml) | Insulin (μU/ml)* | Glucose (mg/100 ml) |
| 1,800                    | 4            | 0.36 ± 0.09           | 21.0 ± 5.6       | 19.5 ± 8.2          | 0.33 ± 0.21           | 29.3 ± 8.9       | 71.0 ± 10.0         |
| 2,200                    | 7            | 0.48 ± 0.06           | 7.6 ± 1.4        | 21.6 ± 2.8          | 0.35 ± 0.06           | 19.2 ± 5.2       | 76.5 ± 9.0          |
| 2,600                    | 27           | 0.48 ± 0.05           | 13.1 ± 1.6       | 17.9 ± 2.4          | 0.52 ± 0.06           | 29.0 ± 7.4       | 118 ± 11.2          |
| 3,000                    | 21           | 0.45 ± 0.03           | 10.0 ± 1.9       | 11.5 ± 1.7          | 0.63 ± 0.06           | 34.5 ± 6.6       | 112 ± 10.2          |
| 3,400                    | 24           | 0.62 ± 0.09           | 15.2 ± 4.6       | 20.2 ± 2.2          | 0.58 ± 0.08           | 34.9 ± 7.2       | 96.2 ± 7.1          |
| 3,800                    | 20           | 0.82 ± 0.10           | 17.5 ± 3.7       | 20.2 ± 2.9          | 0.97 ± 0.15           | 59.0 ± 11.1      | 120 ± 13.9          |
| Correlation coefficient  |              | r = 0.43†             | r = 0.08         | r = 0.07            | r = 0.38†             | r = 0.32†        | r = 0.11            |

\*To convert μU/ml to pmoles/ml, multiply by 0.007.  
†Indicates significant correlation coefficient at p < 0.001.

**Table III.** Correlation of amniotic fluid and cord blood C-peptide, insulin, and glucose levels (mean ± SEM) with weight-gestational age percentile classification

| Weight percentile classification* | Case No. (n) | Amniotic fluid        |                  |                     | Cord blood            |                  |                     |
|-----------------------------------|--------------|-----------------------|------------------|---------------------|-----------------------|------------------|---------------------|
|                                   |              | C-peptide (pmoles/ml) | Insulin (μU/ml)† | Glucose (mg/100 ml) | C-peptide (pmoles/ml) | Insulin (μU/ml)† | Glucose (mg/100 ml) |
| SGA (<10%)                        | 11           | 0.34 ± 0.07           | 12.6 ± 4.9       | 14.9 ± 4.0          | 0.31 ± 0.09           | 17.7 ± 4.3       | 78.4 ± 7.7          |
| AGA (10%-90%)                     | 75           | 0.51 ± 0.03           | 12.7 ± 1.6       | 15.9 ± 1.3          | 0.59 ± 0.04           | 27.4 ± 2.6       | 110 ± 5.9           |
| LGA (>90%)                        | 17           | 0.88 ± 0.09           | 15.0 ± 2.1       | 23.4 ± 3.6          | 0.96 ± 0.18           | 63.8 ± 14.2      | 121 ± 17.6          |
| One-way analysis of variance      |              | f = 10.65‡            | f = 1.29         | f = 2.37            | f = 7.79‡             | f = 5.18§        | f = 17.98‡          |

\*SGA = small for gestational age; AGA = average for gestational age; LGA = large for gestational age.  
†To convert μU/ml to pmoles/ml, multiply by 0.007.  
‡Statistically significant difference at p < 0.001.  
§Statistically significant difference at p < 0.01.

trasound assessment. However, the actual gestational age of infants was evaluated by an experienced pediatrician after the delivery, on the basis of Dubowitz's criteria.<sup>11</sup> Excluded were infants of diabetic mothers and infants who were less than 36 weeks' gestation by pediatric evaluation. In this study, infants were grouped according to weight-gestational age classification into three groups: small for gestational age (SGA), defined as weight below the tenth percentile for gestational age; average for gestational age (AGA), between the tenth and ninetieth percentiles; and large for gestational age (LGA), above the ninetieth percentile. There were 11 SGA, 75 AGA, and 17 LGA infants in this study. Of 25 infants suspected prenatally of IUGR, 13 were above the tenth percentile and were included in the AGA category. A special study form was used to record all clinical information and laboratory data on the mother during the course of pregnancy, labor, and delivery, as well as information on the newborn infant. At amniocentesis, 20 ml of amniotic fluid was obtained for simultaneous determination of the lecithin/sphingomyelin ratio (L/S ratio) and levels of creatinine,

C-peptide, insulin, and glucose. Samples of amniotic fluid for measurement of C-peptide (collected in Trasylol and ethylenediaminetetraacetic acid [EDTA]) and insulin were frozen at -30° C until assayed. At delivery, 12 ml of umbilical cord blood was collected from each patient for measurement of C-peptide (in tubes containing Trasylol and EDTA) insulin, and glucose levels. Serum or plasma was separated immediately and the samples for C-peptide and insulin were frozen in the same manner as described for samples of amniotic fluid. In order to investigate the possible transplacental transport of C-peptide, samples of maternal venous blood, umbilical arterial blood, and umbilical venous blood were collected simultaneously from 20 patients who were delivered of AGA infants. Levels of C-peptide in amniotic fluid and cord blood plasma were measured by radioimmunoassay with the use of M 1230 antiserum.<sup>12</sup> At a suitable dilution, 100 μl of antiserum was incubated with 100 μl of sample (cord blood plasma or amniotic fluid). Twenty hours later, 100 μl of <sup>125</sup>I-tyr-C-peptide (0.04 pmol) was added, and after a second 20 hours of incubation,



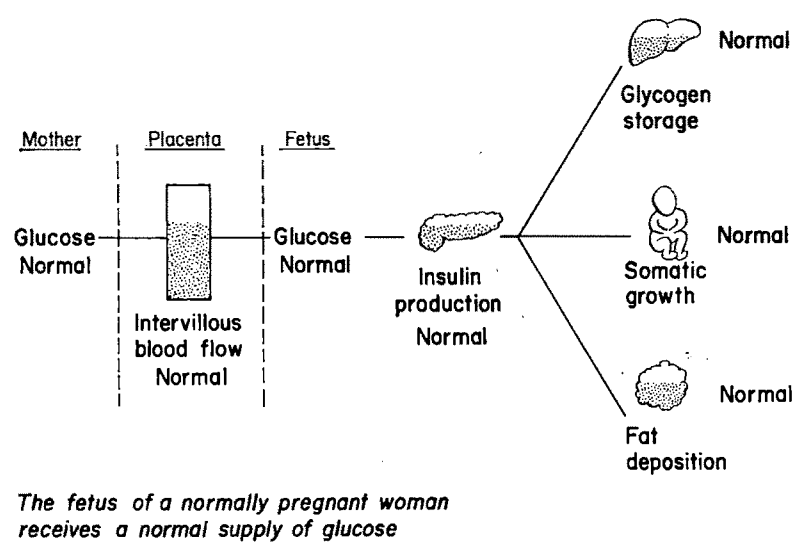


Fig. 4. Insulin regulation of intrauterine growth of AGA fetuses.

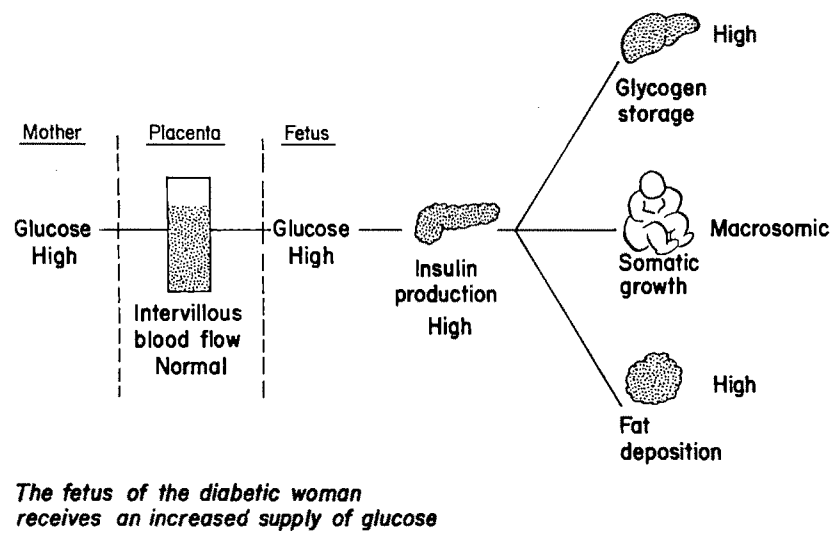


Fig. 5. Insulin regulation of intrauterine growth of LGA fetuses of diabetic mothers.

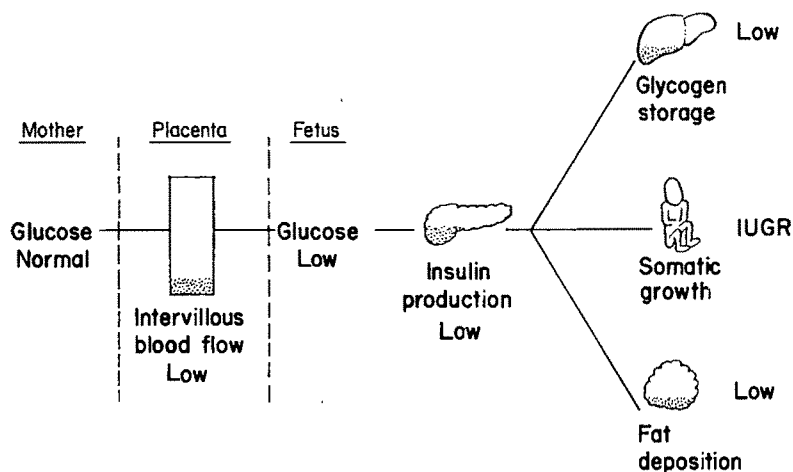
the bound tracer was precipitated with ethanol and counted. Levels of insulin in amniotic fluid and cord blood were measured by a modification of the double-antibody immunoassay method.<sup>13</sup> The concentrations of insulin have been expressed in microunits per milliliter ( $\mu\text{U}/\text{ml}$ ). In order to convert insulin in  $\mu\text{U}/\text{ml}$  to  $\text{pmol}/\text{ml}$  to permit direct comparison with C-peptide, the following calculation can be used:  $\text{Insulin } (\mu\text{U}/\text{ml}) \times 0.007 = \text{insulin } (\text{pmoles}/\text{ml})$ . For determination of glucose, amniotic fluid and cord blood serum were preserved in potassium oxalate and sodium fluoride and assayed with the use of the Beckman glucose analyzer.

Amniotic fluid and cord blood C-peptide (CBCP) levels were compared to placental weight, infant birth

weight, and fetal growth pattern or SGA-AGA-LGA classification. Statistical significance was determined by either correlation coefficient, one-way analysis of variance, or Student's *t* test in different circumstances.

Results

**C-peptide levels in mothers and infants.** As seen in Table I, mean C-peptide levels in umbilical arterial blood; and those in umbilical venous blood were similar, but the cord blood values were much lower than the mean C-peptide levels in maternal venous blood. Insulin does not cross the placenta in human beings.<sup>14</sup> Although the molecular weight of C-peptide (3,000) is about half that of insulin, fetal plasma C-peptide concentrations also appear to be independent of maternal



*The fetus of the hypertensive woman receives a decreased supply of glucose*

Fig. 6. Insulin regulation of intrauterine growth of SGA fetuses.

levels, thus indicating insignificant placental transfer of this peptide.

**Amniotic fluid and cord blood C-peptide levels in relation to placental weight.** Fig. 1 shows the relationship between placental weight and C-peptide levels in both amniotic fluid and cord blood. Neither AFCP levels nor CBCP levels correlated with placental weight.

**Amniotic fluid and cord blood C-peptide levels and infant birth weight.** Fig. 2 shows the correlation between amniotic fluid and cord blood C-peptide levels and infant birth weight. Infant weight was grouped in 400 gram increments. There was a statistically significant correlation of birth weight with both AFCP and CBCP levels.

**Amniotic fluid and cord blood C-peptide levels in relation to fetal growth pattern.** As seen in Fig. 3, the correlation between the fetal growth pattern (or SGA-AGA-LGA classification) and both the AFCP and CBCP levels was highly significant. In each group, the AFCP level was similar to the level in cord blood. The mean values of CBCP were approximately 0.3, 0.6, and 0.9 pmol/ml, respectively. Differences between SGA and AGA and differences between AGA and LGA were both statistically significant. (Statistical comparison by multiple comparison t test: SGA-AGA  $p < 0.025$ ; AGA-LGA  $p < 0.05$ .)

**Amniotic fluid and cord blood insulin levels, glucose levels, and glucose/insulin ratio in relation to infant birth weight and fetal growth pattern.** Table II shows the correlation of amniotic fluid and cord blood C-peptide, insulin, and glucose levels with infant birth weight. Table III shows the correlation of these parameters with weight-gestational age percentile classi-

fication. On the basis of amniotic fluid glucose and insulin levels, the glucose/insulin ratio was calculated for each individual case.

In amniotic fluid, C-peptide was the only parameter that correlated well with the SGA-AGA-LGA classification or the infant birth weight. Neither insulin levels nor the glucose/insulin ratio correlated significantly with infant birth weight or the SGA-AGA-LGA classification. Also, the cord blood glucose levels correlated well with the SGA-AGA-LGA classification.

**Amniotic fluid C-peptide level in relation to L/S ratio.** Among the 103 subjects studied, the correlation between amniotic fluid C-peptide level and L/S ratio was not significant ( $r = 0.21$ ,  $p > 0.05$ ). However, there was a significant difference (Student's t test  $p < 0.001$ ) between the mean C-peptide level in the group having an immature L/S ratio of  $< 2.0$  ( $n = 11$ ; mean  $\pm$  SEM  $0.31 \pm 0.03$  pmoles/ml) and that in the group having a mature L/S ratio of  $\geq 2.0$  ( $n = 92$ ; mean  $\pm$  SEM  $0.52 \pm 0.05$  pmoles/ml).

#### Comment

This study has shown that the AFCP level is a reliable indicator of intrauterine fetal growth, with high levels being present in LGA infants and low levels in SGA infants at term. The advantage of AFCP over CBCP measurements is that the amniotic fluid values are available before delivery, and thus provide a potential prenatal diagnostic tool. Amniotic fluid insulin levels<sup>15</sup> and amniotic fluid glucose/insulin ratio<sup>16</sup> have been reported to be good indicators for distinguishing the fetuses of diabetic mothers from those of normal control mothers. However, in our study of nondiabetic pa-



tients, these two parameters showed large variability and did not correlate with either infant birth weight or fetal growth pattern.

There was no close correlation between cord blood C-peptide and insulin levels. This discrepancy in the molar quantities of these peptides has been noticed in healthy subjects by all investigators.<sup>17</sup> The reasons for this difference depend on (1) the marked differences in hepatic extraction of insulin (40% to 60%) vs. C-peptide (<5%), and (2) the slower peripheral clearance of C-peptide than insulin ( $t_{1/2}$  of approximately 30 minutes for C-peptide vs. 4 to 6 minutes for insulin). Therefore, AFCP is considered to be a better marker than amniotic fluid insulin in reflecting the amount of endogenous fetal insulin secreted.

Our data show relatively high levels of cord blood glucose in all three weight-gestational age percentile groups. This may have been caused by maternal intravenous infusion of glucose during the course of labor and delivery. In the normal condition, fetal blood glucose concentrations are considered to be approximately two thirds of the maternal glucose levels. However, under the circumstances of elevated maternal glucose concentrations secondary to continuous intravenous infusion of glucose, the fetal blood glucose concentrations would be closer to the maternal blood values. In this study, there was a significant difference between the mean cord blood glucose level of the SGA group and that of the AGA group (78 vs. 110 mg/100 ml,  $p < 0.001$ ). Under the influence of maternal infusion of glucose, the mean fetal blood glucose concentration of 110 mg/100 ml of the AGA group is approximately 50 mg/100 ml higher than the level normally seen in nondiabetic AGA infants. Without maternal infusion of glucose, the mean fetal glucose level of the SGA group would be very close to the hypoglycemic level of 30 mg/100 ml (e.g.,  $78 - 50 \text{ mg/100 ml} = 28 \text{ mg/100 ml}$  or  $78 \text{ mg/100 ml} \times \frac{1}{2} = 34 \text{ mg/100 ml}$ ).

We have not studied AFCP levels in preterm pregnancies at  $\leq 35$  weeks' gestation. According to Sosenko and co-investigators,<sup>18</sup> CBCP levels in infants of normal pregnancies increase steadily from less than 34 weeks

to over 37 weeks, in contrast to consistently high CBCP levels before 34 weeks in infants of diabetic mothers. It would be interesting to extend our AFCP study to preterm gestation, and to compare the AFCP levels in term SGA fetuses with the levels in preterm AGA fetuses of similar birth weight. The AFCP levels in the former are expected to be lower than those in the latter.

Figs. 4, 5, and 6 illustrate our hypothesis that insulin is an important factor in the regulation of intrauterine fetal growth. Appropriate secretion of insulin by the AGA fetus leads to normal somatic growth, glycogen storage, and deposition of fat (Fig. 4). The overall supply of glucose stimulates excessive synthesis and secretion of insulin in the fetus of a diabetic mother.<sup>15, 18</sup> This is probably the main mechanism of diabetogenic fetal macrosomia and excessive storage of glycogen and deposition of fat (Fig. 5). In contrast, the low secretion of insulin secondary to chronic fetal hypoglycemia in the SGA fetuses may lead to retardation in somatic growth, a deficient storage of glycogen in the liver and heart, and a lack of subcutaneous deposition of fat. Fig. 6 illustrates this relationship between a chronically low fetal concentration of glucose and a persistently low fetal production of insulin in the SGA fetus. Thus, the data from this study suggest that a persistently low production of insulin in SGA fetuses and high production of insulin in LGA fetuses may lead to the different growth rates observed.

On the basis of our hypothesis of the cause of IUGR, an effective intrauterine therapy, starting at about 30 weeks' gestation, to promote fetal growth and to reduce perinatal morbidity in the SGA fetus could be achieved, if the status of chronic fetal hypoglycemia and low production of insulin is correctable. Bed rest and infusion of hypertonic dextrose,<sup>19</sup> beta-sympathomimetic agents, such as ritodrine, or daily nutritional supplementation with an aim towards increasing the transfer of glucose to the fetal circulation may be helpful in this situation. Finally, AFCP levels measured before and after such treatment may be a useful indicator in the evaluation of the effectiveness of intrauterine therapy.

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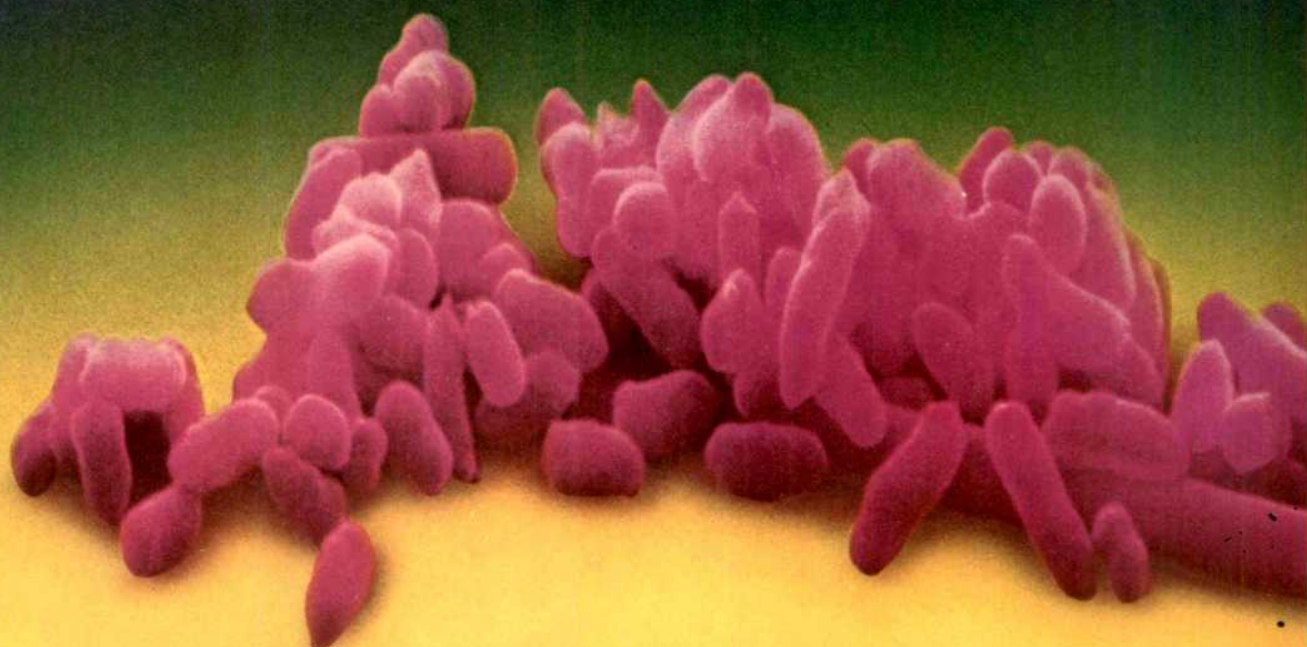
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From Searle-

# **“AN IMPORTANT THERAPEUTIC ADVANCE”\*** against anaerobic infections

\*Sydney M. Finegold, M.D., Professor of Medicine, UCLA School of Medicine; Chief, Infectious Disease Section, Wadsworth VA Medical Center, Los Angeles, California



Scanning electron micrograph of *Bacteroides fragilis* magnified 24,000 times and color enhanced.

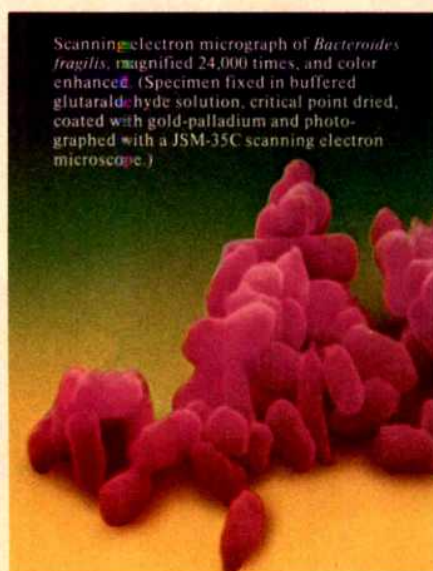


## Important News from Searle

### Announcing

# FLAGYL I.V.<sup>™</sup> (metronidazole HCl), a systemic anaerobicide highly effective against *Bacteroides fragilis*.

Flagyl I.V. is indicated (see indications and warnings) for treatment of serious infections due to *B. fragilis*, which is generally resistant to penicillin G, ampicillin, and most other antibiotics. In a mixed aerobic and anaerobic infection, antibiotics appropriate for the treatment of the aerobic infection should be used in addition to Flagyl I.V.



*B. fragilis* is often pathogenic in infections occurring below the diaphragm, and sometimes is pathogenic in infections above the diaphragm, such as brain and lung abscess, meningitis, aspiration pneumonia, and endocarditis. Flagyl I.V. is indicated in all of these, and has been found to be effective in *B. fragilis* infections not responding to clindamycin, chloramphenicol, or penicillin. See Table 1.

Table 1.\*

| CONDITIONS                                                                                                                     | SUSCEPTIBLE ANAEROBES                                              |                                                                                                                                                                      |                                   |                            |                              |                            |
|--------------------------------------------------------------------------------------------------------------------------------|--------------------------------------------------------------------|----------------------------------------------------------------------------------------------------------------------------------------------------------------------|-----------------------------------|----------------------------|------------------------------|----------------------------|
|                                                                                                                                | <i>Bacteroides</i> species, including the <i>B. fragilis</i> group | <i>Clostridium</i> species                                                                                                                                           | <i>Peptostreptococcus</i> species | <i>Peptococcus</i> species | <i>Fusobacterium</i> species | <i>Eubacterium</i> species |
| INTRA-ABDOMINAL INFECTIONS, including peritonitis, intra-abdominal abscess, liver abscess                                      | ✓                                                                  | ✓                                                                                                                                                                    | ✓                                 | ✓                          |                              | ✓                          |
| SKIN AND SKIN STRUCTURE INFECTIONS                                                                                             | ✓                                                                  | ✓                                                                                                                                                                    | ✓                                 | ✓                          | ✓                            |                            |
| GYNECOLOGIC INFECTIONS, including endometritis, endomyometritis, tubo-ovarian abscess, and postsurgical vaginal cuff infection | ✓                                                                  | ✓                                                                                                                                                                    | ✓                                 | ✓                          |                              |                            |
| BACTERIAL SEPTICEMIA                                                                                                           | ✓                                                                  | ✓                                                                                                                                                                    |                                   |                            |                              |                            |
| BONE AND JOINT INFECTIONS, as adjunctive therapy                                                                               | ✓                                                                  | *Based on Flagyl I.V. <sup>™</sup> (metronidazole HCl)<br>Clinical Studies: Susceptible Anaerobes and Conditions Frequently Caused by One or More of These Organisms |                                   |                            |                              |                            |
| CNS INFECTIONS, including brain abscess and meningitis                                                                         | ✓                                                                  |                                                                                                                                                                      |                                   |                            |                              |                            |
| LOWER RESPIRATORY TRACT INFECTIONS, including pneumonia, empyema, and lung abscess                                             | ✓                                                                  |                                                                                                                                                                      |                                   |                            |                              |                            |
| ENDOCARDITIS                                                                                                                   | ✓                                                                  |                                                                                                                                                                      |                                   |                            |                              |                            |



## Important News from Searle

# FLAGYL I.V.<sup>TM</sup> (metronidazole HCl) is effective against a wide range of anaerobes that often cause serious infections.

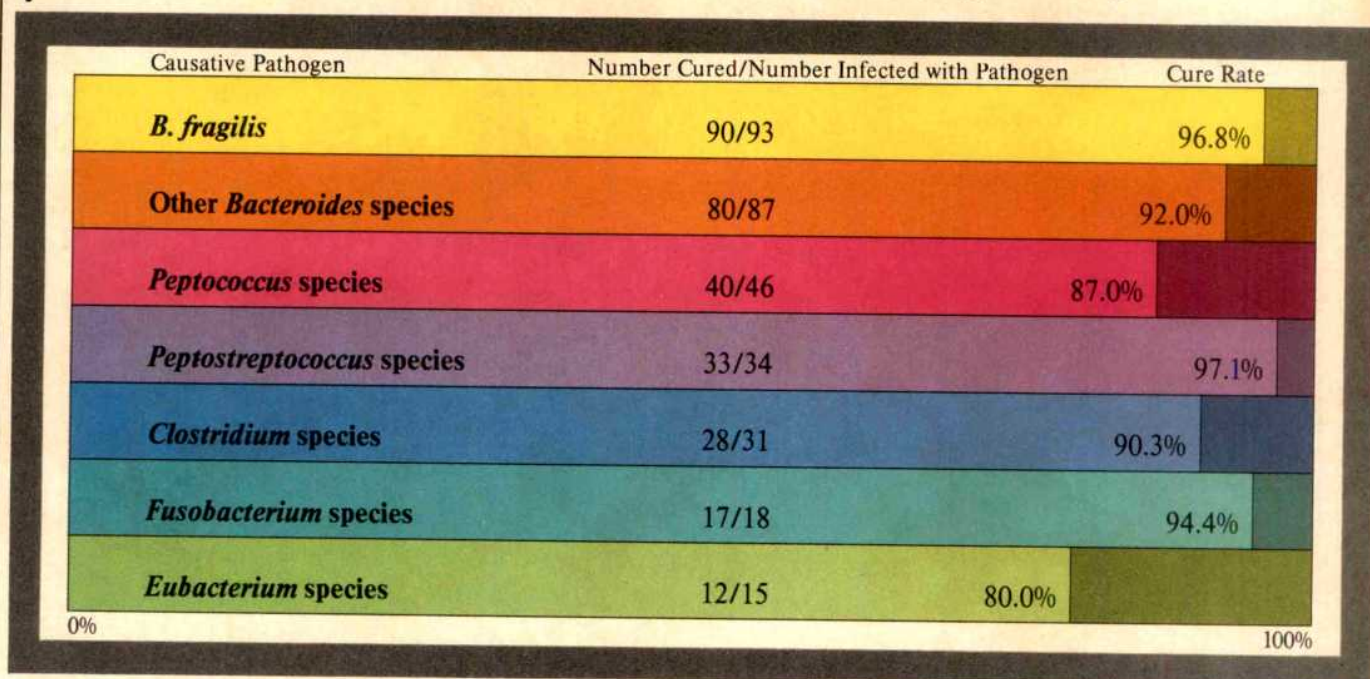
■ Metronidazole is "... consistently bactericidal against anaerobes."<sup>1</sup>

■ Metronidazole readily crosses the blood-brain barrier.

■ Metronidazole achieves excellent tissue penetration.

The chart below\* shows the effectiveness of Flagyl I.V. against major anaerobes in clinical studies involving 180 patients. (The anaerobes and conditions are listed in Table 1.)

Patients Cured with Flagyl I.V.<sup>TM</sup> (metronidazole HCl) by Type of Pathogen



NOTE: The above chart is based on pooled data from two clinical studies representing a total of 180 patients with various kinds of infections, including: intra-abdominal, skin and skin structure, gynecologic, bone and joint, CNS,

and pleuropulmonary infections; bacterial septicemia; and endocarditis. Of the 180 patients, 106 (58.9%) were infected with more than one anaerobe.

## SEARLE

\*Data on file, G. D. Searle & Co.

Please see brief summary of prescribing information on last page of this advertisement.



## Important News from Searle

**In clinical trials with  
FLAGYL I.V.<sup>TM</sup> (metronidazole HCl),  
the frequency and type of adverse  
reactions followed the pattern of  
17 years' previous experience  
with oral metronidazole.**

**FLAGYL I.V.  
is effective and relatively safe.**

- No cases of pseudomembranous colitis have been reported to date with Flagyl I.V.
- No cases of aplastic anemia have been reported.
- Although phlebitis has been reported, this reaction can be avoided or minimized by proper I.V.-admixture and -administration procedures.
- Metronidazole has been shown to be carcinogenic in mice and rats. The relevance of this finding with regard to humans has not been demonstrated. See prescribing information for *Warnings* and *Indications*.

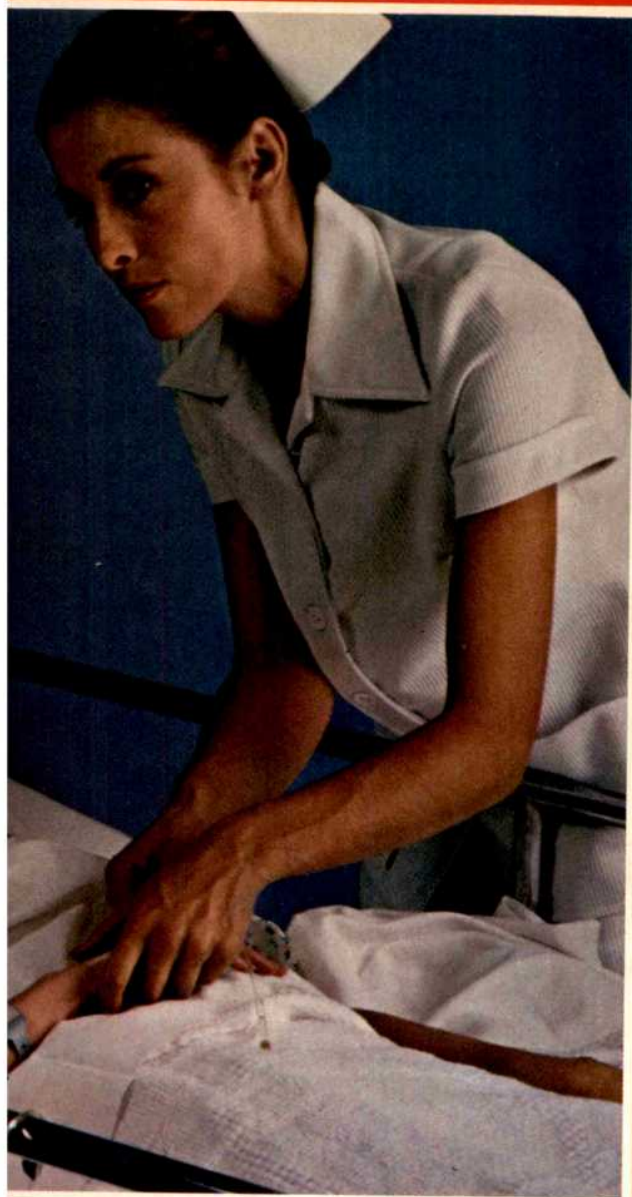
• For information concerning adverse reactions, consult the brief summary of prescribing information on the last page of this advertisement.





## Important News from Searle

**Drug-induced resistance to  
FLAGYL I.V.<sup>TM</sup> (metronidazole HCl)  
has not been documented,  
and the development of  
significant resistance  
appears unlikely to occur.**



■ Although specific anaerobes may be naturally resistant to Flagyl I.V., drug-induced resistance has not been documented.

■ No drug-induced resistance was reported in Flagyl I.V. clinical trials.

■ Oral metronidazole usage (for trichomoniasis and amebiasis), in over 20 million patients and for more than 17 years in the United States alone, has not resulted in the reported development of drug resistance in anaerobic bacteria.

■ Drug-induced resistance appears unlikely to occur because of the mechanism of action of Flagyl I.V.

**Reference:**

1. Finegold, S.M., Antimicrobial Therapy of Anaerobic Infections: A Status Report, *Hosp. Pract.* 14:71-81 (Oct.) 1979.

**SEARLE**

Please see brief summary of prescribing information on next page.



# FLAGYL I.V.<sup>TM</sup> (metronidazole HCl)

## Flagyl I.V.<sup>TM</sup> (metronidazole HCl)

### WARNING

Metronidazole has been shown to be carcinogenic in mice and rats (see *Warnings*). Its use, therefore, should be reserved for serious anaerobic infections where, in the judgment of the physician, the benefit outweighs the possible risk.

**Indications and Usage:** Flagyl I.V. is indicated in the treatment of serious infections caused by susceptible anaerobic bacteria. Indicated surgical procedures should be performed in conjunction with Flagyl I.V. therapy. In a mixed aerobic and anaerobic infection, antibiotics appropriate for the treatment of the aerobic infection should be used in addition to Flagyl I.V.

Flagyl I.V. has been found to be effective in *Bacteroides fragilis* infections resistant to clindamycin, chloramphenicol, and penicillin.

**INTRA-ABDOMINAL INFECTIONS**, including peritonitis, intra-abdominal abscess and liver abscess, caused by *Bacteroides* species including the *B. fragilis* group (*B. fragilis*, *B. distasonis*, *B. ovatus*, *B. thetaiotaomicron*, *B. vulgatus*), *Clostridium* species, *Eubacterium* species, *Peptococcus* species, and *Peptostreptococcus* species.

**SKIN AND SKIN STRUCTURE INFECTIONS** caused by *Bacteroides* species including the *B. fragilis* group, *Clostridium* species, *Peptococcus* species, *Peptostreptococcus* species, and *Fusobacterium* species.

**GYNECOLOGIC INFECTIONS**, including endometritis, endomyometritis, tubo-ovarian abscess, and post-surgical vaginal cuff infection, caused by *Bacteroides* species including the *B. fragilis* group, *Clostridium* species, *Peptococcus* species, and *Peptostreptococcus* species.

**BACTERIAL SEPTICEMIA** caused by *Bacteroides* species including the *B. fragilis* group, and *Clostridium* species.

The following conditions when caused by *Bacteroides* species including the *B. fragilis* group:

- **BONE AND JOINT INFECTIONS**, as adjunctive therapy
- **CNS INFECTIONS**, including meningitis and brain abscess
- **LOWER RESPIRATORY TRACT INFECTIONS**, including pneumonia, empyema, and lung abscess
- **ENDOCARDITIS**

**Contraindications:** Prior history of hypersensitivity to metronidazole or other nitroimidazole derivatives.

**Warnings:** Convulsive seizures and peripheral neuropathy have been reported in patients treated with metronidazole. The appearance of abnormal neurologic signs demands the prompt evaluation of the continuation of the drug.

Metronidazole has shown evidence of carcinogenicity in studies involving chronic, oral administration in mice and rats, but similar studies in hamsters were negative. It has also shown mutagenicity in some *in vitro* assays, but *in vivo* studies did not show a potential for genetic damage.

**Precautions:** Since patients with severe hepatic disease metabolize metronidazole slowly with resultant accumulation of metronidazole and its metabolites in the plasma, doses below those usually recommended should be administered cautiously. Known or previously unrecognized candidiasis may present more prominent symptoms during therapy and requires treatment with a candidicidal agent.

Use with care in patients with evidence of or history of blood dyscrasia. Mild leukopenia has been observed during metronidazole administration; however, no persistent hematologic abnormalities attributable to metronidazole have been observed in clinical studies. Total and differential leukocyte counts are recommended before and after therapy.

Metronidazole has been reported to potentiate the anticoagulant effect of warfarin and other oral coumarin anticoagulants resulting in a prolongation of prothrombin time and this should be considered when Flagyl I.V. is prescribed for patients on such anticoagulant therapy.

Avoid alcoholic beverages during metronidazole therapy.

Metronidazole may interfere with certain chemical analyses for serum glutamic oxalacetic transaminase resulting in decreased values.

Safe use in pregnancy has not been established. Metronidazole crosses the placental barrier and enters the fetal circulation rapidly. This drug should be used during pregnancy only if clearly needed.

Metronidazole is secreted in breast milk in concentrations similar to those found in plasma. If use of Flagyl I.V. is deemed essential, an alternative method of infant feeding should be used.

Safety and effectiveness in children have not been established.

**Adverse Reactions:** Convulsive seizures, nausea, vomiting, abdominal discomfort, diarrhea, unpleasant metallic taste, reversible neutropenia (leukopenia), erythematous rash, pruritus, headache, dizziness, syncope, fever, darkened urine, and thrombophlebitis after intravenous infusion that can be minimized or avoided by not prolonging use of indwelling intravenous catheters. Since persistent peripheral neuropathy has been reported in some patients receiving prolonged metronidazole therapy, patients should be observed carefully if neurologic symptoms occur and a prompt evaluation made of the benefit/risk ratio of the continuation of therapy.

Additional adverse reactions have been reported during treatment with oral metronidazole: anorexia, epigastric distress, constipation; furry tongue, glossitis, and stomatitis sometimes with a sudden overgrowth of *Candida*; flattening of the T-wave in electrocardiographic tracings; vertigo, incoordination, ataxia, confusion, irritability, depression, weakness, insomnia, urticaria, flushing, nasal congestion; dryness of mouth (or vagina or vulva), dysuria, cystitis, polyuria, incontinence, a sense of pelvic pressure, proliferation of *Candida* in the vagina, dyspareunia, decrease of libido, proctitis, and fleeting joint pains.

### Dosage and Administration:

#### Adults:

**Loading Dose**—15 mg/kg infused over one hour (approximately 1 g for a 70-kg adult).

**Maintenance Dose**—7.5 mg/kg infused over one hour every six hours (approximately 500 mg for a 70-kg adult). The first maintenance dose should be instituted six hours following the initial loading dose.

Parenteral therapy may be changed to oral Flagyl<sup>®</sup> (metronidazole) when conditions warrant, based upon the severity of the disease and the response of the patient to Flagyl I.V. treatment. The usual adult oral dosage is 7.5 mg/kg every six hours. A maximum of 4.0 g should not be exceeded during a 24-hour period.

Patients with severe hepatic disease metabolize metronidazole slowly, with resultant accumulation of metronidazole and its metabolites in the plasma. Accordingly, for such patients, doses below those usually recommended should be administered cautiously. Close monitoring of plasma metronidazole levels and toxicity is recommended.

The dose of Flagyl I.V. should not be specifically reduced in anuric patients since accumulated metabolites may be rapidly removed by dialysis.

The usual duration of therapy is 7 to 10 days; however, infections of the bone and joint, lower respiratory tract, and endocardium may require longer treatment.

Flagyl I.V. is to be administered by slow intravenous drip infusion only, either as a continuous or intermittent infusion. I.V. admixtures containing Flagyl I.V. and other drugs should be avoided. If used with a primary intravenous fluid system, the primary solution should be discontinued during infusion. Flagyl I.V. cannot be given by direct intravenous injection (I.V. bolus) because of the low pH (0.5 to 2.0) of the reconstituted product. FLAGYL I.V. MUST BE FURTHER DILUTED AND NEUTRALIZED FOR I.V. INFUSION.

**How Supplied:** Flagyl I.V. (metronidazole HCl) is supplied in single-dose lyophilized vials each containing 500 mg metronidazole equivalent, and 415 mg mannitol.

Searle Pharmaceuticals Inc.  
Box 5110, Chicago, Illinois 60680

OF1

# SEARLE



# OVCON-35<sup>®</sup>

21 peach tablets of norethindrone 0.4 mg and ethinyl estradiol 0.035 mg.  
Each green tablet in the 28-day regimen contains inert ingredients.

**It's safe  
it's effective  
and it's lower dose**



For Brief Summary, see next page.



# Ovcon-35

21 peach tablets of norethindrone 0.4 mg and ethinyl estradiol 0.035 mg. Each green tablet in the 28-day regimen contains inert ingredients.

**Indications and Usage:** Ovcon is indicated for the prevention of pregnancy. Some combination products containing 35 mcg. or less of ethinyl estradiol report slightly higher pregnancy rates than are reported for the higher dose combination products.

**Contraindications:** Oral contraceptives should not be used in women with any of the following conditions: 1) Thrombophlebitis or thromboembolic disorders. 2) A past history of deep vein thrombophlebitis or thromboembolic disorders. 3) Cerebral vascular or coronary artery disease. 4) Known or suspected carcinoma of the breast. 5) Known or suspected estrogen dependent neoplasia. 6) Undiagnosed abnormal genital bleeding. 7) Known or suspected pregnancy.

#### Warnings:

Cigarette smoking increases the risk of serious cardiovascular side effects from oral contraceptive use. Women who use oral contraceptives should be strongly advised not to smoke. Use of oral contraceptives is associated with increased risk of several serious conditions, including thromboembolism, stroke, myocardial infarction, hepatic adenoma, gallbladder disease, hypertension. Practitioners prescribing oral contraceptives should be familiar with the following information relating to these risks.

1) **Thromboembolic Disorders and Other Vascular Problems.** An increased risk of thromboembolic and thrombotic disease associated with use of oral contraceptives is well established. Three principal studies in Great Britain and three in the United States have demonstrated an increased risk of fatal and nonfatal venous thromboembolism and stroke, both hemorrhagic and thrombotic. These studies estimate that users of oral contraceptives are 4 to 11 times more likely than nonusers to develop these diseases without evident cause. In a study of cerebrovascular disorders in women with and without predisposing causes, it was estimated that the risk of hemorrhagic stroke was 2.0 times greater than nonusers and the risk of thrombotic stroke was 4 to 9.5 times greater. An increased risk of myocardial infarction associated with the use of oral contraceptives has been reported. Studies conducted in the United Kingdom found that the greater the number of underlying risk factors for coronary artery disease (cigarette smoking, hypertension, hypercholesterolemia, obesity, diabetes, history of preclampsia toxemia) the higher the risk of developing myocardial infarction, regardless of whether the patient was an oral contraceptive user or not. Oral contraceptives, however, were found to be a clear additional risk factor. In terms of relative risk, it has been estimated that oral contraceptive users who do not smoke (smoking considered a major predisposing condition to myocardial infarction) are about twice as likely to have a fatal myocardial infarction compared to nonusers who do not smoke. Oral contraceptive users who smoke have about a 10- to 12-fold increased risk compared to nonusers who do not smoke. The amount of smoking is a very important factor. British investigators concluded that the risk of thromboembolism including coronary thrombosis is directly related to the dose of estrogen used in oral contraceptives; preparations containing 100 mcg. or more of estrogen were associated with a higher risk of thromboembolism than those containing 50-80 mcg. of estrogen. The relative risk of thromboembolic disease associated with progestin-only oral contraceptives has not been determined. Cases of thromboembolic disease have been reported in women using progestin-only products, and they should not be presumed to be risk-free. The overall excess mortality rate annually from circulatory diseases for oral contraceptive users was estimated to be 20 per 100,000 (ages 15-35, 5/100,000; ages 35-44, 33/100,000; ages 45-49, 140/100,000), the risk being concentrated in older women, in those with a long duration of use, and in cigarette smokers. The highest risk was found in heavy cigarette smokers (15 or more cigarettes per day) who used oral contraceptives and were aged 40 or older. Women who smoke should be advised not to use oral contraceptives. The use of oral contraceptives in women over age 40 with other risk factors is not recommended. The mortality associated with all of the methods of birth control is low compared to the risk of childbirth, with the exception of oral contraceptive users who smoke and are over age 40. The lowest mortality is associated with the condom or diaphragm backed up by early abortion. The risk of thromboembolic and thrombotic disease associated with oral contraceptives increases with age after approximately age 30 and for myocardial infarction is further increased by hypertension, hypercholesterolemia, obesity, diabetes, or history of preclampsia toxemia, and especially by cigarette smoking. The physician and the patient should be alert to the earliest manifestations of thromboembolic and thrombotic disorders (e.g., thrombophlebitis, pulmonary embolism, cerebrovascular insufficiency, coronary occlusion, retinal thrombosis and mesenteric thrombosis). Should any of these occur or be suspected, the drug should be discontinued immediately. If feasible, oral contraceptives should be discontinued at least 4 weeks before surgery of a type associated with an increased risk of thromboembolism or prolonged immobilization. 2) **Ocular Lesions.** There have been reports of neuro-ocular lesions such as optic neuritis or retinal thrombosis associated with the use of oral contraceptives. Discontinue the medication if there is unexplained sudden or gradual, partial or complete loss of vision; sudden onset of proptosis or diplopia; papilledema; or retinal vascular lesions, and institute appropriate diagnostic and therapeutic measures. 3) **Carcinoma.** Long term administration of either natural or synthetic estrogen in certain animal species increases the frequency of carcinoma of the breast, cervix, vagina and liver. In humans, one study investigated the first 21 cases of endometrial adenocarcinoma in women on oral contraceptives reported to a registry. Of those women without predisposing risk factors for this disease, nearly all occurred in women who had used a sequential oral contraceptive. No evidence has been reported suggesting an increased risk of endometrial cancer in users of conventional combination or progestin-only oral contraceptives. No increase in breast cancer in women taking oral contraceptives has been reported although one study reported an increased risk of breast cancer in subgroups of women treated using oral contraceptives with documented benign breast disease. There is at present no confirmed evidence from human studies of an increased risk of cancer associated with oral contraceptives. Close clinical surveillance of all women taking oral contraceptives is essential. In all cases of undiagnosed persistent or recurrent abnormal vaginal bleeding, appropriate diagnostic measures should be taken to rule out malignancy. Women with a strong family history of breast cancer or who have breast nodules, fibrocystic disease or abnormal mammograms should be monitored with particular care if they elect to use oral contraceptives. 4) **Hepatic Adenoma.** Benign hepatic adenomas have been found to be associated with the use of oral contraceptives. One study reported a higher risk associated with oral contraceptive formulations with high hormonal potency. Although benign and rare, hepatic adenomas may rupture and may cause death through intra-abdominal hemorrhage. This has been reported in short term as well as long term users of oral contraceptives. Two studies relate the risk with duration of contraceptive use, the risk being much greater after 4 or more years of oral contraceptive use. While hepatic adenoma is a rare lesion, it should be considered in women presenting abdominal pain and tenderness, abdominal mass, or shock. A few cases of hepatocellular carcinoma have been reported in women taking oral contraceptives. The relationship of these drugs to this type of malignancy is not known at this time. 5) **Use in Pregnancy, Birth Defects in Offspring and Malignancy in Female Offspring.** Fetal abnormalities have been reported to occur in the offspring of women who have taken progestogens and/or estrogens during pregnancy. It has been shown that females exposed in utero to diethylstilbestrol, a nonsteroidal estrogen, have an increased risk of developing in later life a form of vaginal or cervical cancer that is ordinarily extremely rare. A high percentage of such exposed women (30% to 90%) have been found to have epithelial changes of the vagina and cervix. Although these changes are histologically benign it is not known whether this condition is a precursor of vaginal malignancy. Male children so exposed may develop abnormalities of the urogenital tract. Similar data are not available with the use of other estrogens but it cannot be presumed that they would not induce similar changes. The data suggest that the risk of limb reduction defects in exposed fetuses is somewhat less than 1 in 1000 live births. In the past, female sex hormones have been used during pregnancy in an attempt to treat threatened or habitual abortion. There is considerable evidence that estrogens are ineffective for these indications and there is no evidence from well-controlled studies that progestins are effective for these uses. Increases in chromosomal aberrations have been reported in women who become pregnant soon after ceasing

oral contraceptive therapy. Embryos with these anomalies are virtually always spontaneously aborted. Whether there is an overall increase in spontaneous abortion of pregnancies conceived soon after stopping oral contraceptives is unknown. It is recommended that for any patient who has missed two consecutive periods, pregnancy should be ruled out before continuing the contraceptive regimen. If the patient has not adhered to the prescribed schedule, the possibility of pregnancy should be considered at the time of the first missed period and further use of oral contraceptives should be withheld until pregnancy has been ruled out. If pregnancy is confirmed the patient should be apprised of the potential risks to the fetus and the advisability of pregnancy continuation should be discussed in light of these risks. It is also recommended that women who discontinue oral contraceptives with the intent of becoming pregnant use an alternate form of contraception for a period of time before attempting to conceive. Many clinicians recommend three months. Administration of progestin-only or progestin-estrogen combinations to induce withdrawal bleeding should not be used as a test for pregnancy. 6) **Gall Bladder Disease.** Studies have reported an increased risk of surgically confirmed gallbladder disease appearing after one year of oral contraceptive use and doubling of the risk after 4 or 5 years of use. 7) **Carbohydrate and Lipid Metabolic Effects.** A decrease in glucose tolerance has been observed in a significant percentage of patients on oral contraceptives. For this reason, prediabetic and diabetic patients should be carefully observed while receiving oral contraceptives. Increased serum levels of triglycerides and total phospholipids have been observed in oral contraceptive users. The clinical significance of this observation is unknown at this time. 8) **Elevated Blood Pressure.** An increase in blood pressure has been reported in women receiving oral contraceptives. The prevalence of hypertension in oral contraceptive users may be no higher than nonusers in the first year of oral contraceptive use but increases with longer exposure and in the fifth year of use is two and one-half to three times the reported prevalence in the first year. Women who previously had hypertension during pregnancy may be more likely to develop elevation of blood pressure when given oral contraceptives. 9) **Headaches.** The onset or exacerbation of migraine or development of headache of a new pattern which is recurrent, persistent or severe, requires discontinuation of oral contraceptives and investigation of the cause. 10) **Bleeding Irregularities.** Breakthrough bleeding, spotting and amenorrhea are frequent reasons for patients discontinuing oral contraceptives. In breakthrough bleeding, as in all cases of irregular bleeding from the vagina, nonfunctional causes should be borne in mind. In undiagnosed persistent or recurrent abnormal bleeding from the vagina, adequate diagnostic measures are indicated to rule out pregnancy or malignancy. If pathology has been excluded, time or change to another formulation may solve the problem. Changing to an oral contraceptive with a higher estrogen content, while potentially useful in minimizing menstrual irregularity, should be done only if necessary since this may increase the risk of thromboembolic disease. Women with a past history of oligomenorrhea or secondary amenorrhea or young women without regular cycles may have a tendency to remain anovulatory or to become amenorrheic after discontinuation of oral contraceptives. Women with these preexisting problems should be advised of this possibility and encouraged to use other contraceptive methods. 11) **Ectopic Pregnancy.** Ectopics as well as intrauterine pregnancy may occur in contraceptive failures. However, in oral contraceptive failures, the ratio of ectopic to intrauterine pregnancies is higher than in women not using oral contraceptives since the drugs are more effective in preventing intrauterine rather than ectopic pregnancy. The higher ectopic-intrauterine ratio has been reported with both combination products and progestin-only oral contraceptives. 12) **Breast Feeding.** A small fraction of the hormonal agents in oral contraceptives has been identified in the milk of mothers receiving these drugs. The long-range effect to the nursing infant cannot be determined at this time.

**Precautions:** 1) A complete medical and family history should be taken prior to the initiation of oral contraceptives. Examination should include special reference to blood pressure, breasts, abdomen and pelvic organs, including Papanicolaou smear and relevant laboratory tests. As a general rule, oral contraceptives should not be prescribed for longer than 1 year without another physical examination being performed. 2) Under the influence of estrogen-progestogen preparations, preexisting uterine leiomyomata may increase in size. 3) Patients with a history of psychic depression should be carefully observed and the drug discontinued if depression recurs to a serious degree. 4) Because oral contraceptives may cause some degree of fluid retention, conditions which might be aggravated by fluid retention, such as convulsive disorders, migraine syndrome, cardiac or renal insufficiency or asthma require careful observation. 5) Patients with a past history of jaundice during pregnancy have an increased risk of recurrence of jaundice while receiving oral contraceptive therapy. If jaundice develops in any patient receiving such drugs, the medication should be discontinued. 6) Steroid hormones may be poorly metabolized in patients with impaired liver function and should be administered with caution in such patients. 7) Oral contraceptive users may have disturbances in normal tryptophan metabolism which may result in a relative pyridoxine deficiency. The clinical significance of this is yet to be determined. 8) Serum folate levels may be depressed by oral contraceptive therapy. This may complicate subsequent pregnancy with regard to folate deficiency. 9) The pathologist should be advised of oral contraceptive therapy when relevant specimens are submitted. 10) Certain endocrine and liver function tests and blood components may be affected by estrogen-containing oral contraceptives: a. Increased sulfobromophthalen retention. b. Increased prothrombin and factors VII, VIII, IX, and X; decreased antithrombin III; increased norepinephrine-induced platelet aggregability. c. Increased thyroid binding globulin (TBG) leading to increased circulating total thyroid hormone, as measured by protein-bound iodine (PBI),  $T_4$  by column, or  $T_4$  by radioimmunoassay. Free  $T_4$  resin uptake is decreased, reflecting the elevated TBG, free  $T_4$  concentration is unaltered. d. Decreased pregnandiol excretion. e. Reduced response to metyrapone test.

**Information for the Patient:** Detailed Patient Labeling has been prepared for use by the patient and has been made available for distribution by the pharmacist.

**Drug Interactions:** Reduced efficacy and increased incidence of breakthrough bleeding have been associated with concomitant use of rifampin. A similar association has been suggested with barbiturates, phenylbutazone, phenytoin sodium and ampicillin.

**Adverse Reactions:** An increased risk of the following serious adverse reactions has been associated with the use of oral contraceptives (see **Warnings**): thrombophlebitis, pulmonary embolism, coronary thrombosis, cerebral thrombosis, cerebral hemorrhage, hypertension, gall bladder disease, congenital anomalies. There is evidence of an association between the following conditions and the use of oral contraceptives, although additional confirmatory studies are needed: Mesenteric thrombosis; benign hepatomas; neuro-ocular lesions, e.g., retinal thrombosis and optic neuritis. The following adverse reactions have been reported in patients receiving oral contraceptives and are believed to be drug related: Nausea and/or vomiting, usually the most common adverse reactions, occur in approximately 10% or less of patients during the first cycle (Other reactions, as a general rule, are seen much less frequently or only occasionally); gastrointestinal symptoms (such as abdominal cramps and bloating); breakthrough bleeding; spotting; change in menstrual flow; dysmenorrhea; amenorrhea during and after treatment; temporary infertility after discontinuance of treatment; edema; chloasma or melasma which may persist; breast changes (tenderness, enlargement, and secretion); change in weight (increase or decrease); change in cervical erosion and cervical secretion; possible diminution in lactation when given immediately postpartum; cholestatic jaundice; migraine; increase in size of uterine leiomyomata; rash (allergic); mental depression; reduced tolerance to carbohydrates; vaginal candidiasis. The following adverse reactions have been reported in users of oral contraceptives, and the association has been neither confirmed nor refuted: Premenstrual-like syndrome, intolerance to contact lenses, change in corneal curvature (steepening), cataracts, changes in libido, chorea, changes in appetite, cystitis-like syndrome, headache, nervousness, dizziness, hirsutism, loss of scalp hair, erythema multiforme, erythema nodosum, hemorrhagic eruption, vaginitis, porphyria, impaired renal function.

**Acute Overdosage:** Serious ill effects have not been reported following acute ingestion of large doses of oral contraceptives by young children. Overdosage may cause nausea, and withdrawal bleeding may occur in females.

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# Minor (glycosylated) hemoglobins in cord blood of infants of normal and diabetic mothers

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The levels of minor (glycosylated) hemoglobins in maternal blood during labor and in cord blood at delivery were determined in four groups of women: 20 normal, 22 probably normal, 20 Class A diabetic, and eight overt diabetic women. Maternal HbA<sub>1</sub> was not significantly increased in the diabetic patients, thereby reflecting adequate metabolic control. In the cord blood, the percentage of the minor hemoglobin HbF<sub>1c</sub>, which has been shown to be a mixture of acetylated and glycosylated HbF, and the ratios HbF<sub>1c</sub>/HbF and HbA<sub>1</sub>/HbA were significantly increased in fetuses delivered of diabetic mothers. This supports the view that these fetuses are hyperglycemic in utero. However, there was no correlation between cord blood glycosylated hemoglobins and the birth weight ratio, a sensitive indicator of relative changes in the birth weight. (AM. J. OBSTET. GYNECOL. 139:397, 1981.)

SEVERAL chromatographically separable minor hemoglobins (Hbs), namely, A<sub>1a1</sub>, A<sub>1a2</sub>, A<sub>1b</sub>, and A<sub>1c</sub>, are present in the red cell lysate of normal adults.<sup>1</sup> HbA<sub>1c</sub>, which accounts for the major portion of the minor Hbs, has a hexose moiety (1-amino,1-deoxy fructose) attached to the amino-terminal valine of the  $\beta$  chains through the formulation of a Schiff base and subsequent Amadori rearrangement.<sup>1</sup> HbA<sub>1a1</sub> and HbA<sub>1a2</sub> were shown to be adducts of HbA with fructose-1,6-diphosphate and glucose-6-phosphate, respectively.<sup>1</sup>

Fetal hemoglobin (HbF) is the predominant type of Hb in the fetus. With the use of Biorex 70 chromatography, several minor Hbs, namely, pre-F<sub>1</sub> (F<sub>1a+b</sub>) and F<sub>1</sub> (F<sub>1c</sub>), were separated from cord blood red cell lysate.<sup>2</sup> Recently, the HbF<sub>1a+b</sub> component has been further separated into HbF<sub>1a1</sub>, HbF<sub>1a2</sub>, and HbF<sub>1b</sub>.<sup>3</sup> Chemical

analysis of the minor components and in vitro glycosylation studies<sup>2, 3</sup> indicated that HbF<sub>1a1</sub> and HbF<sub>1a2</sub> have been formed by the modification of  $\gamma$  chains of HbF by reacting with fructose-1,6-diphosphate and glucose-6-phosphate, respectively.<sup>3</sup> HbF<sub>1c</sub> contained both glycosylated (glucose adduct) and acetylated derivatives of HbF.<sup>2, 3</sup> HbF<sub>1b</sub> is a glycohemoglobin; the mechanism of its formation is not known at present.

The levels of HbA<sub>1c</sub> and HbA<sub>1</sub> (HbA<sub>1a+b+c</sub>) were shown to be increased in patients with diabetes mellitus.<sup>4-6</sup> Moreover, the levels of these Hbs were reported to be higher in diabetic than in normal pregnant women.<sup>7, 8</sup> However, it is not known whether the levels of glycosylated Hbs (HbF<sub>1a+b+c</sub> and HbA<sub>1</sub>) are increased in fetal blood in diabetic pregnancies. Fetal macrosomia in diabetic pregnancies has been attributed to hyperinsulinemia, which occurs in response to fetal hyperglycemia that is secondary to maternal hyperglycemia.<sup>9</sup> Although this theory is commonly accepted, direct evidence is still lacking.<sup>10</sup> This is because it is not possible to determine fetal blood glucose levels during pregnancy. Cord blood glycosylated Hbs may reflect the average levels of fetal blood glucose in the last weeks of pregnancy, so that measurement of them could be useful in this regard.

In this study, the levels of the different minor Hbs were measured in the cord blood of infants delivered from normal as well as diabetic (both gestational and

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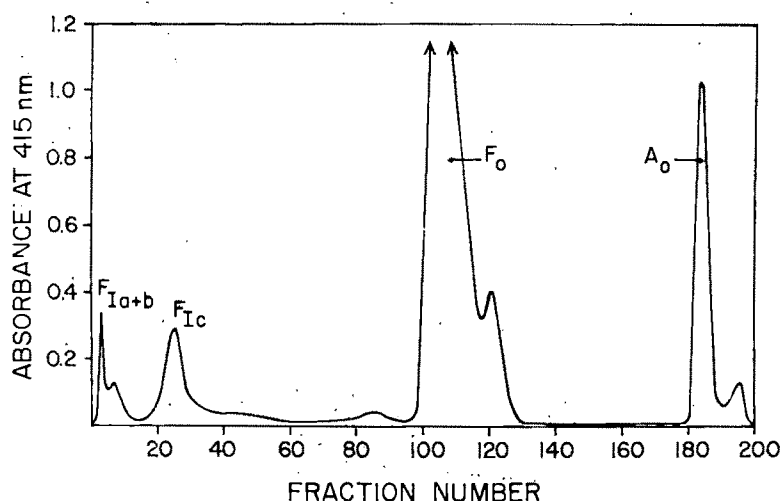


Fig. 1. Biorex 70 column chromatographic separation of hemoglobins present in red cell lysate from a sample of cord blood.

overt) women. The purpose of this study was twofold: first, to determine whether there are differences in the relative amounts of minor (glycosylated) Hbs between infants of diabetic mothers and normal infants; and secondly, to correlate the levels of glycosylated Hbs with the birth weight and to examine this correlation relative to the hyperglycemia-hyperinsulinemia theory.

#### Subjects and methods

Seventy pregnant women who consented to participate in this study were classified into four groups, as follows. *G1*, Twenty normal women with no historical factors suggestive of diabetes and a normal glucose screening test, i.e., blood glucose  $<130$  mg/dl one hour after a glucose load of 50 gm.<sup>11</sup> *G2*, Twenty-two probably normal women who had either historical factors suggestive of diabetes mellitus and a normal glucose screening test or an abnormal glucose screening test but a normal oral glucose tolerance test.<sup>11</sup> *G3*, Twenty women with an abnormal glucose tolerance test,<sup>11</sup> i.e., White's Class A diabetics. *G4*, Eight women with insulin-dependent (overt) diabetes, of whom five were in Class B and three were in Class C.

Diabetic Class A patients were treated by diet. Overt diabetic patients were, in addition, treated with insulin. The aim was to maintain the patients in "euglycemia" as closely as possible.<sup>12</sup> Ultrasonography was used for determination of the gestational age in all of the diabetic and in some of the normal pregnant women. The gestational ages of all of the infants were confirmed by neonatal assessment based on the Dubowitz scoring system,<sup>13</sup> and the birth weight ratio (BWR) was calculated for all of them. The actual birth weight was divided by

the fiftieth percentile weight for the corresponding week of gestation as determined from Colorado's intrauterine growth charts.<sup>14</sup> Since the BWR is considered to be a more accurate indicator of relative changes in birth weight, it was used instead of the actual birth weight in the different analyses in this study.

Samples of blood (7 ml) were obtained from the mothers at the time of their admission in labor, and from the cord (3 to 5 ml) at delivery. The samples were collected in ethylenediaminetetraacetic acid (EDTA) tubes and stored at 4° C. Within 24 hours, hemolysates were prepared from saline-solution-washed red cells by osmotic lysis with water, shaking with  $\text{CCl}_4$ , and subsequent centrifugation to remove cellular debris.<sup>15</sup> Samples containing abnormal Hbs or Hb-Bart's, as detected by starch gel electrophoresis at pH 9.0,<sup>15</sup> were excluded from the study. Each hemolysate was dialyzed immediately against the appropriate developer used for subsequent chromatographic analyses. Red cell lysates were chromatographed on 1 by 25 cm Biorex 70 columns with the use of continuously increasing sodium phosphate gradient at pH 6.7 according to the procedure of Abraham and associates.<sup>2</sup> About 30 mg of Hb was applied on each column and developed at room temperature for 3 to 4 days. A flow rate of 12 to 15 ml per hour was maintained and 20 minute fractions were collected. The absorbancy of the effluent fractions was read at 415 nm, and the percentages of the Hb components were calculated on the basis of these absorbance readings. Fig. 1 shows the chromatographic separation of Hbs from red cell lysate of a normal newborn infant (cord blood). In selected patients, total HbA<sub>1</sub> was quantitated in cord blood by means of a dieth-



Table I. Clinical data on the study patients

|                                  | Normal, G1<br>(N = 20)       | Probably normal,* G2<br>(N = 22) | Diabetics                    |                              | Significant differences†  |
|----------------------------------|------------------------------|----------------------------------|------------------------------|------------------------------|---------------------------|
|                                  |                              |                                  | Class A, G3<br>(N = 20)      | Overt, G4<br>(N = 8)         |                           |
| Age (yr)                         | 20.7 ± 5.5‡<br>(16-38)       | 22.4 ± 6.1<br>(15-41)            | 27.2 ± 5.3<br>(19-37)        | 29.3 ± 7.9<br>(18-42)        | G3 and G4 > G1 and G2     |
| Parity                           | 0.7 ± 0.8<br>(0-2)           | 1.5 ± 1.8<br>(0-8)               | 1.7 ± 1.6<br>(0-6)           | 1.5 ± 1.2<br>(0-3)           | NS                        |
| Gestational age at delivery (wk) | 38.8 ± 1.4<br>(36-41)        | 38.1 ± 2.3<br>(32-41)            | 37.7 ± 2.9<br>(30-41)        | 36.8 ± 1.9<br>(34-40)        | NS                        |
| Birth weight (gm)                | 3,203 ± 532<br>(2,100-3,950) | 3,137 ± 529<br>(1,810-4,343)     | 3,364 ± 757<br>(1,420-4,480) | 3,517 ± 723<br>(1,980-4,100) | NS                        |
| Birth weight ratio§              | 1.03 ± 0.15<br>(0.77-1.25)   | 1.07 ± 0.11<br>(0.9-1.36)        | 1.17 ± 0.22<br>(0.75-1.61)   | 1.26 ± 0.22<br>(0.89-1.56)   | G4 > G1 and G2<br>G3 > G1 |

\*See text for criteria of inclusion in this group.  
†Tukey's multiple comparison test, p = <0.05.  
‡Mean ± SD and (range).  
§The ratio between the actual birth weight and the fiftieth percentile weight at the same gestational age.

Table II. Comparison of the percentages\* of different hemoglobin components in cord blood of different groups of patients studied

|                       | G1 (N = 20)               | G2 (N = 22)               | G3 (N = 20)               | G4 (N = 8)                | Significant differences† |
|-----------------------|---------------------------|---------------------------|---------------------------|---------------------------|--------------------------|
| HbF <sub>ia+b</sub> % | 5.3 ± 1.0‡<br>(3.8-7.2)   | 5.2 ± 1.0<br>(3.7-7.1)    | 4.9 ± 2.2<br>(1.3-9.6)    | 5.4 ± 1.9<br>(2.4-7.5)    | NS                       |
| HbF <sub>ic</sub> %   | 9.1 ± 1.3<br>(5.4-11.2)   | 9.7 ± 2.1<br>(6.4-14.5)   | 10.4 ± 2.1<br>(7.0-14.5)  | 11.5 ± 1.6<br>(9.0-13.5)  | G4 > G1                  |
| HbF <sub>i</sub> %§   | 14.4 ± 1.9<br>(9.5-16.8)  | 14.8 ± 2.7<br>(10.4-21.6) | 15.3 ± 3.2<br>(10.8-23.2) | 16.8 ± 2.5<br>(12.0-20.3) | NS                       |
| HbF <sub>0</sub> %    | 69.2 ± 4.5<br>(60.1-76.1) | 69.4 ± 6.9<br>(54.3-79.5) | 66.6 ± 7.2<br>(55.8-76.1) | 71.3 ± 4.2<br>(65.8-77.4) | NS                       |
| HbA <sub>1</sub> %    | 2.1 ± 0.5<br>(1.5-2.7)    | 2.2 ± 0.5<br>(1.7-2.9)    | 2.5 ± 0.4<br>(2.0-3.0)    | 3.3 ± 1.5<br>(1.0-5.0)    | NS                       |
| HbA <sub>0</sub> %    | 14.6 ± 4.4<br>(8.3-27.0)  | 14.2 ± 6.6<br>(5.2-27.7)  | 16.7 ± 6.6<br>(7.4-30.6)  | 11.6 ± 3.6<br>(5.4-17.1)  | NS                       |

\*Expressed as a percentage of the total Hb in the hemolysate from the cord blood.  
†Tukey's multiple comparison test, p = <0.05.  
‡Mean ± SD and (range).  
§HbF<sub>i</sub> is the sum of HbF<sub>ia+b</sub> + HbF<sub>ic</sub>.  
||Determined by DEAE cellulose chromatography in five of each of G1, G2, and G3 patients and in six of G4 patients.

ylaminoethyl (DEAE) cellulose chromatographic procedure,<sup>16, 17</sup> and in the maternal blood by means of a microcolumn procedure.<sup>\*6</sup>

The results were analyzed by a standard computer program. The statistical tests performed were Tukey's multiple comparison test, and Pearson's linear regression.<sup>18</sup>

Results

The pertinent clinical data for the patients in the different groups are given and compared in Table I. The diabetic patients (G3 and G4) were significantly older than the normal women (G1 and G2). There

were no significant differences in parity between the study groups. The diabetic women underwent delivery slightly earlier than did the normal women, but the differences were not significant. Nineteen large-for-gestational-age (LGA) babies were delivered: 5/42 of normal women, 8/20 of class A patients, and 6/8 of overt diabetic women. Infants of diabetic mothers (G3 and G4) were slightly heavier than those of G1 and G2 mothers, but the differences were insignificant. However, the BWR was significantly higher for the infants of G4 mothers than for those of both G1 and G2 mothers, and also for the infants of G3 mothers than for those of G1 mothers (Table I).

HbA<sub>1</sub> levels in the samples of blood obtained during labor were slightly higher in G4 patients (7.7% ± 2.1%)

\*Quick-Sep, Isolab, Inc., Akron, Ohio.

**Table III.** Comparison of the ratio of minor to total hemoglobins in cord blood from the different groups of patients studied

|                                                          | G1<br>(N = 20)            | G2<br>(N = 22)            | G3<br>(N = 20)            | G4<br>(N = 8)             | Significant<br>differences* |
|----------------------------------------------------------|---------------------------|---------------------------|---------------------------|---------------------------|-----------------------------|
| $\frac{\text{HbF}_{\text{Ia+b}}}{\text{HbF}} \times 100$ | 6.3 ± 1.1‡<br>(4.7-8.7)   | 6.2 ± 1.2<br>(4.5-9.6)    | 6.0 ± 2.8<br>(1.7 ± 12.0) | 6.1 ± 2.1<br>(2.6-8.5)    | NS                          |
| $\frac{\text{HbF}_{\text{Ic}}}{\text{HbF}} \times 100$   | 10.9 ± 1.4<br>(7.8-13.0)  | 11.4 ± 2.0<br>(7.4-16.3)  | 12.7 ± 2.4<br>(8.1-17.1)  | 13.1 ± 1.8<br>(10.1-16.1) | G3 and G4 > G1              |
| $\frac{\text{HbF}_{\text{I}}}{\text{HbF}} \times 100$    | 17.1 ± 2.0<br>(13.1-20.9) | 17.6 ± 2.8<br>(12.8-24.2) | 18.8 ± 4.0<br>(13.2-29.1) | 19.1 ± 2.9<br>(13.4-22.6) | NS                          |
| $\frac{\text{HbA}_1}{\text{HbA}} \times 100$             | 12.4 ± 3.6<br>(9.2-17.9)  | 9.6 ± 3.1<br>(4.6-12.2)   | 14.2 ± 3.5<br>(9.1-18.6)  | 23.9 ± 13.7<br>(6.3-38.4) | G4 > G2                     |

\*Tukey's multiple comparison test,  $p = <0.05$ .  
†HbF is the total fetal hemoglobin, i.e.,  $\text{HbF}_0 + \text{HbF}_1$ .  
‡Mean ± SD and (range).  
§HbF<sub>I</sub> is the sum of  $\text{HbF}_{\text{Ia+b}} + \text{HbF}_{\text{Ic}}$ .  
||HbA is the total adult hemoglobin, i.e.,  $\text{HbA}_1 + \text{HbA}_0$ . Both fractions were determined by DEAE cellulose chromatography in five G1, G2, and G3 patients and in six G4 patients.

**Table IV.** Relation of birth weight ratio to selected minor hemoglobin levels in the different study groups\*

|                                                        | G1                 | G2                | G3                | G4               |
|--------------------------------------------------------|--------------------|-------------------|-------------------|------------------|
| Maternal HbA <sub>1</sub> %                            | 0.502†<br>(N = 15) | 0.652<br>(N = 20) | 0.246<br>(N = 15) | 0.932<br>(N = 6) |
| HbF <sub>Ic</sub> %                                    | 0.194<br>(N = 20)  | 0.528<br>(N = 22) | 0.569<br>(N = 20) | 0.066<br>(N = 8) |
| $\frac{\text{HbF}_{\text{Ic}}}{\text{HbF}} \times 100$ | 0.774<br>(N = 20)  | 0.360<br>(N = 22) | 0.375<br>(N = 20) | 0.227<br>(N = 8) |
| $\frac{\text{HbA}_1}{\text{HbA}} \times 100$           | 0.124<br>(N = 5)   | 0.601<br>(N = 5)  | 0.210<br>(N = 5)  | 0.089<br>(N = 6) |
| HbF <sub>Ic</sub> + HbA <sub>1</sub> %                 | 0.286<br>(N = 5)   | 0.260<br>(N = 3)  | 0.994<br>(N = 5)  | 0.198<br>(N = 6) |

\*Pearson's linear regression.  
†P value.

than in either G1 (6.4% ± 0.7%) or G2 patients (6.9% ± 1.4%), but this increase was statistically insignificant. The percentages of the various minor Hb components, and the ratios of the latter to total HbF or HbA in the cord blood from the different groups of patients in the study are presented in Tables II and III, respectively. The ratios were calculated in order to obviate differences that may have resulted from the varying proportions of HbF and HbA in the various specimens examined. With Tukey's multiple comparison test, the only statistically significant differences ( $p < 0.05$ ) were: the higher percentage of F<sub>Ic</sub> fraction in G4 than in G1 (11.49% ± 1.59% vs. 9.09% ± 1.34%), the increased ratio  $\frac{\text{HbF}_{\text{Ic}}}{\text{HbF}} \times 100$  in G3 and G4 over G1, and the increased ratio  $\frac{\text{HbA}_1}{\text{HbA}} \times 100$  in G4 over G2 (Tables II and III).

By regression analysis, there was no correlation between maternal HbA<sub>1</sub> levels (during labor) and the BWR. Also none of the correlations between the various cord blood Hb components and BWR was statistically significant ( $p < 0.05$ ) in any of the groups (Table IV). The analysis was then repeated with reference to the LGA infants only. Still no significant differences were noticed.

**Comment**

The four groups of pregnant women studied were comparable in parity. Diabetes mellitus is expected to complicate pregnancies at a relatively older age, as observed in our patients. The absence of significant differences in the gestational age at delivery reflects our policy of awaiting spontaneous onset of labor in most cases of Class A diabetes and not inducing labor in



overt diabetic patients unless there is a fetal indication.<sup>12</sup> The incidence of LGA babies was comparable to that reported recently.<sup>19</sup> There was a higher incidence of LGA babies and the mean birth weights were higher in the diabetic patients. The differences were insignificant when the actual birth weights were compared, but significant differences were demonstrated when the BWRs were compared. This confirms that BWR more accurately reflects relative changes in birth weight.

Glycosylation is a slow, nonenzymatic, posttranscriptional, irreversible reaction of Hb with glucose (and other phosphorylated glycolytic intermediates) that continues throughout the lifespan of the red blood cells.<sup>1, 20</sup> The product of such reaction of glucose with adult Hb is HbA<sub>1c</sub>. Its concentration was shown to be proportional only to the time-averaged blood glucose to which the red cells have been exposed during their lifetime.<sup>1, 20</sup> Recently, it has been reported that, when erythrocytes from cord blood were incubated with an elevated concentration of glucose, HbF decreased; whereas hemoglobins focusing at isoelectric points near those expected for the corresponding glycosylated derivatives appeared in proportionately increased amounts,<sup>21</sup> thus supporting the presence of a glycosylated HbF component. More recently, a minor HbF component (HbF<sub>1c</sub>) was separated and shown to be a mixture of glycosylated and acetylated HbF.<sup>2, 3</sup>

HbA<sub>1c</sub> and, to a lesser extent, HbA<sub>1a+b</sub> levels are increased in diabetes mellitus both in nonpregnant<sup>4-6</sup> and in pregnant individuals.<sup>7, 8, 19, 22-25</sup> This is expected because the mean maternal blood glucose levels are increased in diabetic patients. Glucose is easily transported across the placenta by facilitated diffusion. It is commonly believed, without direct evidence in the human subject, that there is fetal hyperglycemia in diabetic pregnancies.

We found that HbF<sub>1c</sub> percentage was significantly increased in the cord blood of infants of overt diabetic mothers (G4), and that the  $\frac{\text{HbF}_{1c}}{\text{HbF}} \times 100$  ratio was significantly increased in infants of all diabetic mothers (G3 and G4). HbA<sub>1</sub> percentage was also increased ( $3.3\% \pm 1.5\%$  vs.  $2.1\% \pm 0.5\%$ ), but the increase was not statistically significant. This was probably due to the small number (five) of cord samples in which HbA<sub>1</sub> percentage was measured. However, when the ratio  $\frac{\text{HbA}_1}{\text{HbA}} \times 100$  was compared, it was found to be significantly increased. This may have been due to the relatively small percentage of HbA in cord blood, so that the increase became significant when the percentage of HbA that was glycosylated rather than the abso-

lute percentage of HbA<sub>1</sub> were compared. The increase in HbF<sub>1c</sub> and in these ratios indicates a greater degree of glycosylation of both HbF and HbA in the fetuses of diabetic mothers. Glycosylated Hb levels do not fluctuate with temporary changes in blood glucose levels, but represent the average blood glucose level over the previous 4 to 8 weeks.<sup>5, 19, 22-25</sup> These findings were reported in relation to the glycosylation of HbA, but most likely are also true for HbF, because HbF and HbA have been shown to react with glucose in vitro at about the same rate and extent.<sup>26</sup> Therefore, it is reasonable to expect that cord blood glycosylated Hbs will reflect fetal blood glucose levels in the last weeks of pregnancy.

This study is the first demonstration of an increased level of glycosylated Hbs in fetuses of diabetic mothers, a finding that substantiates the widely accepted view that these fetuses are hyperglycemic. The percentage increase in HbF<sub>1c</sub> was not so great as the percentage increase in HbA<sub>1</sub> usually found in adult diabetic patients.<sup>6</sup> This was probably due to the fact that most of our patients were well controlled, as evidenced by the nearly normal maternal HbA<sub>1</sub> levels at the time of labor. In fact, the highest levels of HbF<sub>1c</sub> in G4 were found in three infants whose mothers were referred to our center only shortly before labor, so that there was not enough time to achieve a control as good as that in the other patients. An alternative explanation is that HbF<sub>1c</sub> is a combination of acetylated and glycosylated components,<sup>2, 3</sup> and only the latter component is expected to increase. The lack of a significant increase in HbF<sub>1a+b</sub> percentage in fetuses delivered from diabetic mothers parallels the very slight increase in HbA<sub>1a+b</sub> percentage levels reported in adult diabetic patients.<sup>6</sup>

Fetal macrosomia is a characteristic feature of diabetic pregnancy, and is believed to be the result of the growth-promoting influence of insulin, which is secreted in excess by the fetal pancreatic  $\beta$  cells in response to fetal hyperglycemia secondary to maternal hyperglycemia.<sup>9</sup> If this theory is valid, the degree of macrosomia should correlate with fetal and maternal levels of blood glucose. Widness and associates<sup>22, 23</sup> reported that the BWR correlates well with maternal HbA<sub>1</sub> levels, and that one can predict the birth weight from the HbA<sub>1</sub> value. In our previous study,<sup>8</sup> we did not confirm this finding, and postulated that this lack of correlation was due to a long interval between the measurement of HbA<sub>1</sub> and delivery (9 weeks); we suggested that the measurement of maternal HbA<sub>1</sub> at the time of labor may evidence this correlation. HbA<sub>1</sub> level at this time will reflect average blood glucose levels over the last 8 weeks of pregnancy, the period during which most of the increase in fetal weight and most of the

deposition of fat occur. In the present study, we still did not find a correlation between maternal HbA<sub>1c</sub> during labor and BWR in either the normal or diabetic groups. Other investigators also failed to find a correlation between maternal HbA<sub>1c</sub> and birth weights.<sup>19, 25</sup>

Our expectation was that the level of glycosylated Hbs in cord blood might be more significantly related to BWR, but such a correlation could not be demonstrated. This may have been due to the fact that most of our diabetic patients were well controlled, with a low incidence of macrosomia that may have masked any such correlation. It would be interesting to expand this

study but to limit the enrollment to patients who are not under such strict metabolic control. On the other hand, this lack of correlation supports the concept that additional factors, including the availability of other substrates, such as free fatty acids, triglycerides, and amino acids, probably play an important role in the determination of the degree of macrosomia as much as does the hyperglycemia itself.<sup>10</sup>

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# Bladder versus renal bacteriuria during pregnancy: Recurrence after treatment

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Localization of bacteriuria has been shown to correlate with the pattern of recurrence after treatment. The immunofluorescent technique was used to localize infection in 233 pregnant women with asymptomatic bacteriuria to determine whether this would identify those who were at greater risk for recurrence. In both an indigent and a military population, the incidence of renal bacteriuria was 42%. Regardless of the site of infection, after one course of short-term (10-day) or long-term (21-day) antimicrobial therapy, almost two thirds of these women were abacteriuric for the remainder of gestation. Women given short-term treatment were more likely to have a recurrence within 2 weeks of completion of therapy than were women given long-term therapy ( $P < 0.001$ ). Moreover, these early recurrences were more frequent in women given short-term treatment for renal bacteriuria ( $P < 0.05$ ). Conversely, recurrences 6 or more weeks after completion of therapy, and regardless of site of infection, were more common in women given long-term treatment ( $P < 0.01$ ). Although the timing of recurrence varied significantly in relation to duration of treatment and site of infection, the ultimate risk of recurrence was not related to either. The conclusion is that localization of asymptomatic bacteriuria does not contribute to the management of pregnant women, since overall recurrence rates are independent of the site of infection. (AM. J. OBSTET. GYNECOL. 139:403, 1981.)

IN THE TWENTY YEARS since Kass<sup>1</sup> emphasized the relationship of asymptomatic bacteriuria during pregnancy to acute antepartum pyelonephritis, screening for bacteriuria has become an important aspect of prenatal care. As a result, approximately two thirds of cases of antepartum pyelonephritis are preventable.<sup>2</sup> Currently, most acute renal infections develop in

women who do not seek prenatal care, or who develop bacteriuria after a sterile urine culture, or in those with recurrent bacteriuria after therapy. The importance of this latter group is illustrated by the threefold decrease in antepartum pyelonephritis observed when frequent culturing of urine was performed to detect recurrence in pregnant women treated for bacteriuria.<sup>3</sup>

With localization by ureteral catheterization or bladder washout, it was shown that bacteriuria during pregnancy is from the kidney in approximately half of cases and from the bladder in the remainder.<sup>4, 5</sup> With invasive localization, and serotyping of bacteria, the response to therapy was studied in nonpregnant patients with chronic bacteriuria, and it was shown that early recurrences after treatment were usually caused by renal bacteriuria.<sup>6-8</sup> In contrast, patients with bladder bacteriuria usually had late recurrences. Furthermore,

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**Table I.** Bacteria isolated and site of involvement in 233 pregnant women with asymptomatic bacteriuria

| Bacteria                     | No. of isolates (%) |         |         |                            |         |         |
|------------------------------|---------------------|---------|---------|----------------------------|---------|---------|
|                              | Parkland Hospital   |         |         | Wilford Hall USAF Hospital |         |         |
|                              | Total               | Renal   | Bladder | Total                      | Renal   | Bladder |
| <i>Escherichia coli</i>      | 126 (83)            | 49      | 76      | 58 (73)                    | 26      | 32      |
| <i>Klebsiella pneumoniae</i> | 16 (11)             | 12      | 4       | 14 (16)                    | 7       | 7       |
| Other*                       | 10 (6)              | 3       | 8       | 9 (11)                     | 1       | 8       |
| Total                        | 152 (100)           | 64 (42) | 88 (58) | 81 (100)                   | 34 (42) | 47 (58) |

\**Proteus* sp., *Enterobacter* sp., and *Citrobacter* sp.

**Table II.** Bacteriuria eradicated for remainder of pregnancy after one course of therapy

|                       | No. of women (%) |             |          |
|-----------------------|------------------|-------------|----------|
|                       | Renal            | Bladder     | Total    |
| Parkland (N = 152)    | 38/64 (59)       | 53/88 (60)  | 91 (60)  |
| Wilford Hall (N = 81) | 22/34 (65)       | 33/47 (70)  | 55 (68)  |
| Total (N = 233)       | 60/98 (61)       | 86/135 (63) | 146 (63) |

these studies showed that renal bacteriuria recurred more frequently than bladder bacteriuria, and that a longer course of therapy was required to eradicate renal infection. These results raise the possibility that detection of asymptomatic renal bacteriuria during pregnancy may be of value in predicting the risk of recurrence, and, therefore, minimize the threat of pyelonephritis.

In 1974, Thomas and associates<sup>9</sup> described the fluorescent antibody test for localization of bacteriuria, and Jones and associates<sup>10</sup> confirmed the accuracy of the technique. This method uses direct immunofluorescence to visualize antibody complexed with bacteria in the urine, and the presence or absence of antibody-coated bacteria localizes infection to the kidney or bladder, respectively. In the present study, we used this simple noninvasive method to localize bacteriuria in asymptomatic pregnant women to determine whether detection of renal bacteriuria might improve clinical management. In this regard, the frequencies and patterns of recurrent infection after long-term and short-term therapy were compared in women with renal bacteriuria and those with bladder bacteriuria.

**Material and methods**

The present report is the result of a prospective collaborative study of women who presented for prenatal care at Parkland Memorial Hospital, Dallas, Texas, and Wilford Hall United States Air Force Medical Center, San Antonio, Texas. The periods of study were from 1974 to 1976 at Wilford Hall and 1976 to 1978 at Parkland. Women were considered for inclusion in the

study at their first prenatal visit if their pregnancy was less than 28 weeks, they were free of urinary tract symptoms, and two consecutive urine cultures had 100,000 or more of a single uropathogen (same species) per milliliter of urine obtained by the clean-voided technique. The fluorescent antibody-coated bacteria test was performed as described by Thomas and associates<sup>9, 11</sup> and was considered to be positive if 25% or more bacteria demonstrated fluorescence. Women with equivocal or unsatisfactory tests were excluded.

**Initial treatment of asymptomatic bacteriuria.** Women at Parkland were given nitrofurantoin, 100 mg at bedtime for 10 days, and were reappointed at intervals consonant with standard obstetric care. Women at Wilford Hall were treated empirically for 21 days with one of the following drugs given four times daily: ampicillin, 250 mg (n = 54); sulfisoxazole, 1,000 mg (n = 10); nitrofurantoin, 100 mg (n = 16); or cephalixin, 250 mg (n = 1). These women were seen at 2-week intervals. At both institutions, a clean-catch specimen of urine was obtained for culture at each subsequent visit, and the women were carefully questioned to assure compliance.

**Response to initial therapy.** Recurrence of bacteriuria was classified as either a relapse or a reinfection depending on the time interval from completion of initial therapy to recurrence. Early recurrence, that is, recurrence within 2 weeks after cessation of therapy, and due to the same species, was considered to be a relapse.<sup>12</sup> Women who had sterile urine after completion of therapy, but who 6 or more weeks later again developed bacteriuria (late recurrence), were considered to have a reinfection.<sup>13</sup> Serotyping of bacteria was not done.

**Statistical methods.** Statistical evaluation of results was performed by means of chi-square contingency table analysis and Fisher's exact test of probability.

**Results**

Enrolled in the study were 233 pregnant women with asymptomatic bacteriuria; 152 were from Parkland Hospital, and 81 were from Wilford Hall Hospital. Both groups were demographically similar when com-



**Table III.** Recurrence of asymptomatic bacteriuria: Relapses and reinfections in relation to duration of therapy and site of bacteriuria

|                                             | Relapses (%) |            |             | Reinfections (%) |            |             |
|---------------------------------------------|--------------|------------|-------------|------------------|------------|-------------|
|                                             | Renal        | Bladder    | Total       | Renal            | Bladder    | Total       |
| Parkland Hospital, 10-day therapy (N = 129) | 16/55 (29)   | 10/74 (14) | 26/129 (20) | 2/39 (5)         | 10/64 (16) | 12/103 (12) |
|                                             | P < 0.05     |            | P < 0.001   |                  |            | P < 0.01    |
| Wilford Hall, 21-day therapy (N = 81)       | 2/34 (6)     | 1/47 (2)   | 3/81 (4)    | 10/32 (31)       | 13/46 (28) | 23/78 (29)  |

pared for age, parity, gestational age at enrollment, and past history of urinary tract infections. Most of the women from Parkland were indigent, and 48% were black. All patients from Wilford Hall were active-duty personnel or military dependents, and 24% were black. The mean duration of culture surveillance was 17.6 weeks at Parkland and 19.8 weeks at Wilford Hall.

**Bacterial isolates and results of localization.** *Escherichia coli* was the initial isolate from 184 (79%) of the 233 women (Table I). Next most common was *Klebsiella pneumoniae* (13%), and the remainder included *Proteus* sp, *Enterobacter* sp, and *Citrobacter* sp. With the exception of *K. pneumoniae*, there were no statistical differences in the frequency of species isolated from women at either hospital, nor between women with renal bacteriuria and women with bladder bacteriuria. At Parkland Hospital, 12 of 16 isolates of *K. pneumoniae* were from women with renal bacteriuria. This is significant ( $P < 0.05$ ) when compared to women with bladder bacteriuria due to the same organism.

#### Response to treatment.

**Eradication of bacteriuria.** One course of antimicrobial therapy was effective in eradicating bacteriuria for the remainder of pregnancy in two thirds of the women (Table II). This was true regardless of the site of infection (bladder or kidney) or the duration of initial therapy (10 or 21 days). No differences in cure were seen when the various regimens used for 21 days were compared. There were no adverse reactions to any antimicrobial agent, except for mild nausea occasionally.

**Pattern of recurrence: Relapse vs. reinfection.** The incidence of recurrences classified as relapses or reinfections after one course of therapy and their relationship to the site of bacteriuria are presented in Table III. At Parkland Hospital, 129 of the 152 women enrolled in the study had additional culturing of urine at intervals permitting classification as defined by the protocol. The other 23 women were followed in the prenatal clinic and underwent delivery at Parkland; two eventually were hospitalized for antepartum pyelonephritis. All women at Wilford Hall had additional culturing of urine at intervals frequent enough to permit classification as defined.

At Parkland Hospital, 26 of 129 women had a urine specimen which was again positive for the same species within 2 weeks after cessation of therapy, and the condition was thus classified as a relapse. In these women who were given treatment for 10 days, a relapse was twice as likely in those with renal bacteriuria as in those with bladder bacteriuria ( $P < 0.05$ ). In contrast, of the 81 women at Wilford Hall given treatment for 21 days, only three (4%) demonstrated a relapse after initial therapy.

Of the other 103 women at Parkland, 12 with intervening sterile cultures after initial therapy developed reinfection. This type of recurrence was found in 16% of women with bladder bacteriuria compared to 5% in those with renal bacteriuria, but this difference was not statistically significant ( $P = 0.095$ ). The incidence of reinfection in the 78 women from Wilford Hall was almost identical: 31% of those with renal bacteriuria and 28% of those with bladder bacteriuria were subsequently identified as having this type of recurrence.

When the patterns of recurrence, namely, relapse versus reinfection, were compared between the two populations, significant differences were found. Women from Parkland given treatment for 10 days were five times more likely to have a relapse as a recurrence than women from Wilford Hall ( $P < 0.001$ ). Conversely, those women from Wilford Hall who were given 21 days of treatment had a 2.5 times greater likelihood of having a reinfection ( $P < 0.01$ ).

#### Comment

Localization of bacteriuria has been reported to be a useful guide for selecting appropriate therapy.<sup>5-8</sup> Using ureteral catheterization, Turck and associates<sup>6, 8</sup> showed that recurrences after treatment for renal bacteriuria are usually relapses, whereas recurrences after treatment for bladder bacteriuria are usually reinfections.<sup>6-8</sup> They further demonstrated that those infections which were relapses were more likely to respond to prolonged (6-week) chemotherapy that was unnecessary for eradication of reinfection. Later, conflicting data were presented by Kincaid-Smith and Fair-

ley,<sup>14</sup> who demonstrated a similar relapse rate (12%) after either a 2-week or 6-week regimen. Other evidence that localization of bacteriuria may have clinical implications comes from studies which indicate that bladder bacteriuria in nonpregnant patients can be eradicated in many cases by topical application of antimicrobial agents or by one dose of intramuscular kanamycin.<sup>5, 15</sup>

The present study was undertaken to determine whether localization of bacteriuria in asymptomatic pregnant women is of any clinical value. In this regard, three questions were asked. (1) Does the risk of recurrence after initial therapy differ if the bacteriuria is renal in origin? (2) Is the pattern of recurrence, defined as relapse or reinfection, related to the site of bacteriuria? (3) Will a longer course of treatment reduce these recurrences, and, if so, does it do so in women with renal or bladder bacteriuria?

Data now presented show that, regardless of the site of infection, or the duration of therapy, approximately two thirds of women treated for bacteriuria will be free from infection for the remainder of gestation. When the patterns of recurrence were analyzed as a *relapse* or a *reinfection*, differences were found when both the site of bacteriuria and the duration of therapy were compared. Women from Parkland Hospital who were treated for 10 days were more likely to have a relapse than a reinfection. Moreover, relapses were significantly more common in women with renal bacteriuria. In contrast, women from Wilford Hall who were given treatment for 21 days seldom had a relapse,

but the incidence of reinfection was increased significantly. These findings are similar to those reported by Turck and associates,<sup>6, 8</sup> and indicate that a relapse after short-term therapy suggests renal infection, whereas a reinfection suggests bladder involvement. Moreover, the efficacy of long-term therapy in obviating relapses due to renal bacteriuria was similar to the experiences of Turck and colleagues.

The results indicate that neither localization of bacteriuria with the fluorescent antibody test nor longer treatment will reduce the risk of a recurrence. Identification of the site of bacteriuria followed by manipulation of duration of therapy merely changes the pattern of recurrence, but not the ultimate risk of recurrence. The need for continued surveillance in these women is emphasized, since Fairley and associates<sup>16</sup> convincingly showed that antepartum pyelonephritis is as likely to result from untreated bladder bacteriuria as from untreated renal bacteriuria. Only two of the 233 women in this study developed pyelonephritis, and this attests to benefits that accrue from continued surveillance, with retreatment given as necessary.

The tests for antibody-coated bacteria in women from Wilford Hall Medical Center were performed in the laboratory of Dr. Virginia Thomas, at the University of Texas Health Sciences Center, San Antonio, Texas. We are indebted to the obstetric clinic nursing staffs and obstetric and gynecology resident physicians at both institutions. We also thank Mrs. Fran Mitchell and Mrs. Barbara Oldstein for expert help in preparation of the manuscript.

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# The fetal and maternal catecholamine response to insulin-induced hypoglycemia in the rat

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This study was undertaken to evaluate the catecholamine response of the fetal rat to insulin-induced hypoglycemia in the mother. Twenty-four Sprague-Dawley rats at day 21 of gestation were used. Hypoglycemia was induced with regular insulin; then at timed intervals, the rats were anesthetized with pentobarbital. When the rats were completely unresponsive, the abdomen was opened, the fetuses were exteriorized, and fetal blood was obtained by sectioning the axillary vessels. Maternal blood was obtained from the vena cava. Plasma glucose was measured by the glucose oxidase technique. Norepinephrine, epinephrine, and dopamine were measured by radioenzymatic assay. Maternal animals demonstrated a fall in glucose to a nadir of  $23 \pm 0.7$  mg/dl (mean  $\pm$  SEM) and a significant rise in plasma epinephrine to a maximum of  $2,548.0 \pm 1,071.2$  pg/ml. Fetal glucose fell to a nadir of  $23.8 \pm 3.3$  mg/dl (mean  $\pm$  SEM) and fetal catecholamines demonstrated a rapid significant rise: norepinephrine rose to a maximum of  $5,077.3 \pm 932.6$  pg/ml; epinephrine rose to a maximum of  $4,295.8 \pm 538.9$  pg/ml; dopamine rose to a maximum of  $869 \pm 203.3$  pg/ml. The fetal correlation coefficients were  $r = -0.56$  ( $p < 0.01$ ) for plasma glucose compared to norepinephrine and  $r = -0.58$  ( $p < 0.01$ ) for plasma glucose compared to epinephrine. The maternal correlation coefficient for plasma glucose compared to epinephrine was  $r = -0.54$  ( $p < 0.01$ ). These data demonstrate that the near-term fetal rat is able to respond significantly to hypoglycemia with a rapid output of catecholamines. (AM. J. OBSTET. GYNECOL. 139:407, 1981.)

FETAL METABOLISM depends on an uninterrupted supply of fuel substrates across the placenta from maternal sources. The data available suggest that glucose is the main fetal fuel; however, there also appears to be some fetal utilization of amino acids, lactate, and possibly ketone bodies.<sup>1, 2</sup> Fetal glucose is provided by the facilitated diffusion of glucose across the placenta.<sup>2, 3</sup> Because of the absence of an active transport mechanism against a concentration gradient, fetal glucose levels are dependent on a higher maternal glucose concentration. Hypoglycemia in the mother will, therefore, result in hypoglycemia in the fetus (see references 14 and 30). This is an important problem for the insulin-dependent diabetic patient. Because of the

therapeutic attempts to produce normoglycemia during pregnancy, episodes of hypoglycemia frequently occur in these patients.

Surplus substrate is stored in late fetal life in the form of glycogen, protein, and triglycerides.<sup>4-6</sup> The fetus appears to be unable to utilize these substrate deposits for the production of endogenous glucose. Glucose-6-phosphatase (G-6-PASE) and phosphorylase, both enzymes that are necessary for glycogenolysis, have been found to be deficient in the fetal liver.<sup>7-10</sup> Hepatic gluconeogenesis is believed not to occur either, because of the deficient levels of phosphoenolpyruvate carboxykinase (PEPCK) and pyruvate carboxylase.<sup>7, 11, 12</sup>

Although glucagon and catecholamines have been demonstrated to induce fetal glycogenolytic and gluconeogenic enzyme activity,<sup>7, 13</sup> their role in the fetal response to hypoglycemia is unclear. With the experimental reduction of fetal monkey glucose levels to values as low as 13 mg/dl, Epstein and associates<sup>14</sup> were unable to elicit an output of fetal glucagon. Multiple authors have demonstrated a fetal catecholamine response to stress, such as hypoxia, in both experimental animals and humans<sup>15-19</sup>; however, no data are avail-

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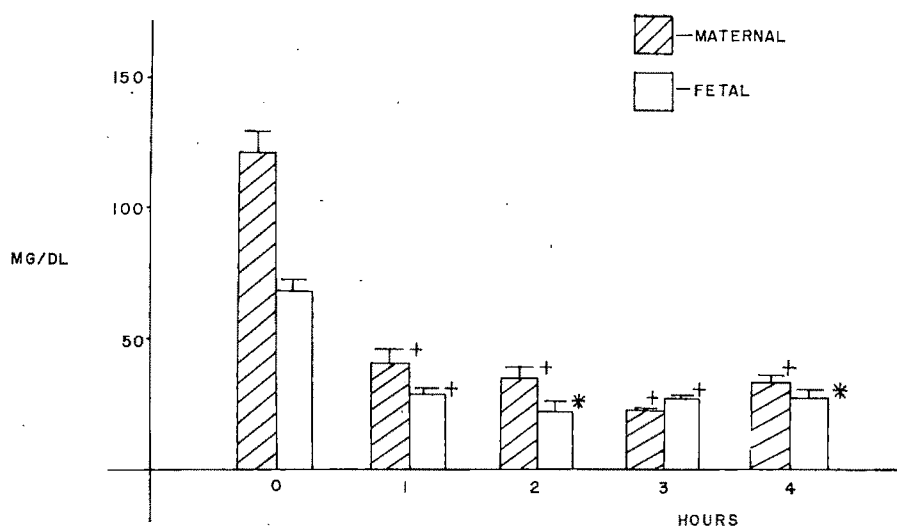


Fig. 1. Maternal and fetal rat plasma glucose concentration (mean  $\pm$  SEM) at control and hourly intervals after maternal injection with 30 U/kg of regular insulin. \* $p < 0.001$  and + =  $p < 0.01$  compared to control values.

Table I. Maternal rat response

| Time    | Glucose (mg/dl)        | NE (pg/ml)                   | EPI (pg/ml)                   |
|---------|------------------------|------------------------------|-------------------------------|
| Control | 117.2 $\pm$ 5.7<br>(6) | 588.8 $\pm$ 185.4<br>(5)     | 217.8 $\pm$ 123.3<br>(5)      |
| 1 hour  | 39.0 $\pm$ 4.1*<br>(6) | 650.0 $\pm$ 101.3<br>(4)     | 659.3 $\pm$ 183.7<br>(4)      |
| 2 hours | 32.8 $\pm$ 5.3*<br>(4) | 1,074.5 $\pm$ 278.1<br>(4)   | 2,123.5 $\pm$ 536.7†<br>(4)   |
| 3 hours | 23.0 $\pm$ 0.7*<br>(4) | 2,906.8 $\pm$ 1,247.2<br>(4) | 2,548.0 $\pm$ 1,071.2†<br>(4) |
| 4 hours | 31.5 $\pm$ 3.9*<br>(4) | 2,432.8 $\pm$ 1,123.5<br>(4) | 1,663.8 $\pm$ 420.7†<br>(4)   |

Mean values  $\pm$  SEM in control animals and at hourly intervals 1 through 4 hours after maternal insulin injection. Numbers in parentheses represent numbers of maternal rats sampled. All values not so indicated were not significantly different compared to control values.

\* $p < 0.01$  compared to control values.

† $p < 0.05$  compared to control values.

able on the fetal catecholamine response to hypoglycemia.

This study was undertaken to evaluate the maternal and fetal catecholamine response to acute insulin-induced hypoglycemia in Sprague-Dawley rats.

#### Material and methods

Twenty-four Sprague-Dawley rats with timed pregnancies and their fetuses were used in this study. On day 21 of gestation the studies were performed. To avoid diurnal variation, all animals were studied between 10 AM and 2 PM. The pregnant rats weighed between 300 and 350 grams; each contained eight to 12 fetuses.

Hypoglycemia was induced by injecting 30 U/kg of regular (short-acting) insulin subcutaneously into the

maternal hip area. Control animals were treated in an identical fashion; however, insulin was not injected. Treated animals were killed at 1-hour intervals 1 to 4 hours after insulin injection. Approximately 15 minutes before the animals were killed, they were injected with 75 mg/kg of pentobarbital for anesthesia. Once the animals were completely unresponsive, usually requiring 15 minutes from the time of barbiturate injection, the maternal abdomen was opened and the uterine horns were exteriorized. As rapidly as possible, the fetuses were dissected individually out of the uterus and sacs. With the umbilical cord and placental circulation remaining intact, fetal blood samples were obtained by sectioning the fetal axillary vessels and collecting the blood in heparinized glass pipettes. The time required from the opening of the maternal abdo-



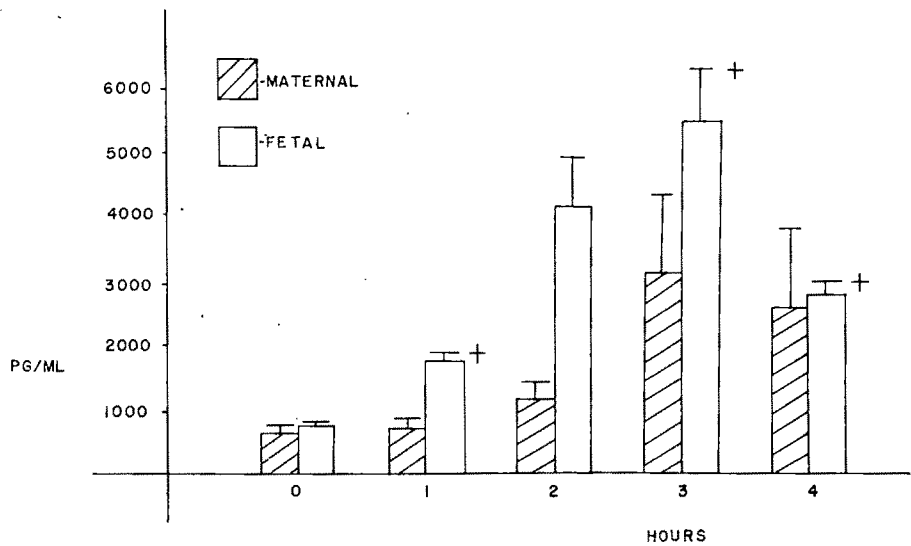


**Fig. 2.** Maternal and fetal rat plasma NE levels (mean  $\pm$  SEM) in control animals and at hourly intervals after maternal injection with regular insulin. + =  $p < 0.01$  compared to control values. Others not significantly different.

men until all fetal and maternal blood samples were obtained was 10 to 15 minutes. Only the first five to six fetuses in each litter were sampled.

Immediately after fetal blood samples were obtained, maternal samples were obtained by drawing blood into a heparinized syringe from the inferior vena cava. Maternal animals continued to breathe spontaneously and have cardiac activity during the time that blood specimens were obtained.

Blood specimens for fetal catecholamine assay were obtained by pooling blood from three fetuses in an iced tube containing 1.2 mg of glutathione and 1.8 mg of ethyleneglycol-bis( $\beta$ -aminoethyl ether)N,N'-tetraacetic acid (EGTA) per milliliter of blood. Blood for fetal glucose determination was obtained from individual fetuses and placed into iced microcentrifuge tubes. Maternal catecholamine and glucose samples were obtained from individual animals.

Catecholamine samples were centrifuged at 2,500 rpm for 5 minutes; the plasma was separated and stored at  $-70^{\circ}\text{C}$  until the assay was performed. All assays were performed within 2 weeks of obtaining the specimens. Glucose samples were likewise centrifuged to obtain plasma; glucose determinations were then performed immediately.

Maternal and fetal glucose determinations were performed by the glucose oxidase technique by means of a Beckman glucose analyzer. The 10  $\mu\text{l}$  plasma specimens were assayed in duplicate.

Catecholamine determinations were performed by means of a radioenzyme assay (Cat a Kit, Upjohn Diag-

**Table II.** Fetal rat responses

| Time    | Glucose (mg/dl)         | NE (pg/ml)                   | EPI (pg/ml)                  |
|---------|-------------------------|------------------------------|------------------------------|
| Control | 68.2 $\pm$ 2.9<br>(11)  | 667.6 $\pm$ 60.3<br>(21)     | 374.9 $\pm$ 69.8<br>(21)     |
| 1 hour  | 27.3 $\pm$ 2.5*<br>(13) | 1,606.3 $\pm$ 144.7*<br>(24) | 1,052.3 $\pm$ 79.3†<br>(21)  |
| 2 hours | 23.8 $\pm$ 3.3‡<br>(10) | 3,909.8 $\pm$ 765.7<br>(12)  | 3,140.8 $\pm$ 499.4*<br>(12) |
| 3 hours | 26.4 $\pm$ 1.8*<br>(8)  | 5,077.3 $\pm$ 932.6*<br>(12) | 4,295.8 $\pm$ 538.9*<br>(12) |
| 4 hours | 27.4 $\pm$ 2.2‡<br>(8)  | 2,631 $\pm$ 138.1*<br>(12)   | 3,888.3 $\pm$ 340.1*<br>(12) |

Mean values  $\pm$  SEM in control animals and at hourly intervals 1 through 4 hours after maternal insulin injection. Number in parentheses represent numbers of fetuses sampled.

\* $p < 0.01$  compared to control values.

† $p < 0.05$  compared to control values.

‡ $p < 0.001$  compared to control values.

nostic) according to the technique described by Passon and Peuler.<sup>20</sup> Epinephrine (EPI), norepinephrine (NE), and dopamine were simultaneously converted into their  $^3\text{H}$ -methoxy derivatives with utilization of catechol-o-methyl transferase (COMT) and ( $^3\text{H}$ -methyl)-S-adenosyl-L-methionine ( $^3\text{H}$ -SAM). The  $^3\text{H}$ -methoxy derivatives were purified with extraction techniques, then separated by thin-layer chromatography.  $^3\text{H}$ -3-Methoxytyramine (dopamine derivative) was extracted into toluene/isoamyl alcohol/Liquifluor (700:300:50, v:v:v) and counted.  $^3\text{H}$ -Normetanephrine (NE derivative) and  $^3\text{H}$ -metanephrine (EPI derivative) were separately oxidized with 4% sodium metaperiodate,

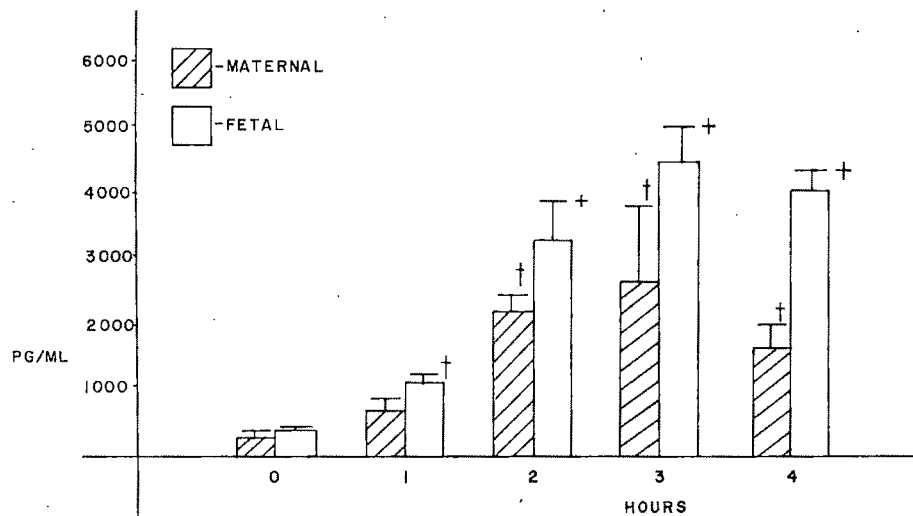


Fig. 3. Maternal and fetal rat plasma EPI levels (mean  $\pm$  SEM) in control animals and at hourly intervals after maternal injection with regular insulin. + =  $p < 0.01$  and  $\dagger = p < 0.05$  compared to control values. Others not significantly different.

forming  $^3\text{H}$ -vanillins. These were extracted into toluene/Liquifluor (1000:50, v:v), and counted. Beta counting was performed by means of a Searle Mark II liquid scintillation counter. This assay is specific for catecholamines; sensitivity is 2 to 5 pg for EPI and NE and 15 to 20 pg for dopamine per 50  $\mu\text{l}$  sample. All 50  $\mu\text{l}$  samples were assayed in duplicate.

Maternal and fetal results were grouped into control and 1-, 2-, 3-, and 4-hour categories for the four variables measured in this study (i.e., glucose, NE, EPI, and dopamine). Mean values  $\pm$  SEM were determined and compared to control values by the Wilcoxon Rank Sum Test to determine significant differences. Linear correlation coefficients were calculated to determine the relationship between glucose and catecholamine levels in both the maternal and fetal rat.

### Results

Control maternal animals had a nonfasting plasma glucose level of  $117.2 \pm 5.7$  mg/dl ( $\pm$  SEM). By 1 hour after insulin injection, the maternal plasma glucose level fell to  $39.0 \pm 4.1$  mg/dl ( $p < 0.01$ ) (Table I). The maternal glucose level continued to fall so that by 2 hours after insulin injection it had reached  $32.8 \pm 5.3$  mg/dl. Maternal glucose reached its lowest level,  $23.0 \pm 0.7$  mg/dl, at 3 hours, then rose at 4 hours to a value of  $31.5 \pm 3.9$  mg/dl. As demonstrated in Fig. 1, the maternal plasma glucose level was initially 72% higher than the fetal plasma glucose level; however, in response to insulin, the maternal-fetal difference decreased dramatically so that by 3 hours the maternal

glucose level was lower than the level observed in the fetus.

Fetal glucose in the control animals was  $68.2 \pm 2.9$  mg/dl ( $\pm$  SEM). In response to maternal hypoglycemia (Table II), the fetal plasma glucose levels fell significantly by 1 hour to  $27.3 \pm 2.5$  mg/dl ( $p < 0.01$ ). The fetal glucose level reached its nadir at 2 hours with a level of  $23.8 \pm 3.3$  mg/dl. Fetal glucose levels then plateaued at 3 hours at  $26.4 \pm 1.8$  mg/dl and at 4 hours at  $27.4 \pm 2.2$  mg/dl. The fetal glucose level (Fig. 1) fell to one third of the control level, in contrast to the maternal glucose value which fell to 20% of the control value.

The maternal NE mean value in the control animals was  $588.8 \pm 185.4$  pg/ml ( $\pm$  SEM). Although maternal NE values rose to  $2,906.8 \pm 1,247.2$  pg/ml by 3 hours, this change was not statistically significant.

In the fetuses the control NE value was  $667.6 \pm 60.3$  pg/ml ( $\pm$  SEM); NE then demonstrated a rapid, significant rise (Table II) by 1 hour after maternal insulin injection to  $1,606.3 \pm 144.7$  pg/ml ( $p < 0.01$ ). Fetal NE continued to rise so that by 2 hours it had reached  $3,909.8 \pm 765.7$  pg/ml and eventually peaked by 3 hours at  $5,077.3 \pm 932.6$  pg/ml. Fetal NE then declined to  $2,631.0 \pm 138.1$  pg/ml at 4 hours. As demonstrated in Fig. 2, fetal NE is significantly ( $p < 0.01$ ) higher than maternal values at 1 hour after insulin injection. NE levels in the fetus were observed to reach the highest level of all catecholamines measured (Table II).

The plasma EPI mean value in the control maternal



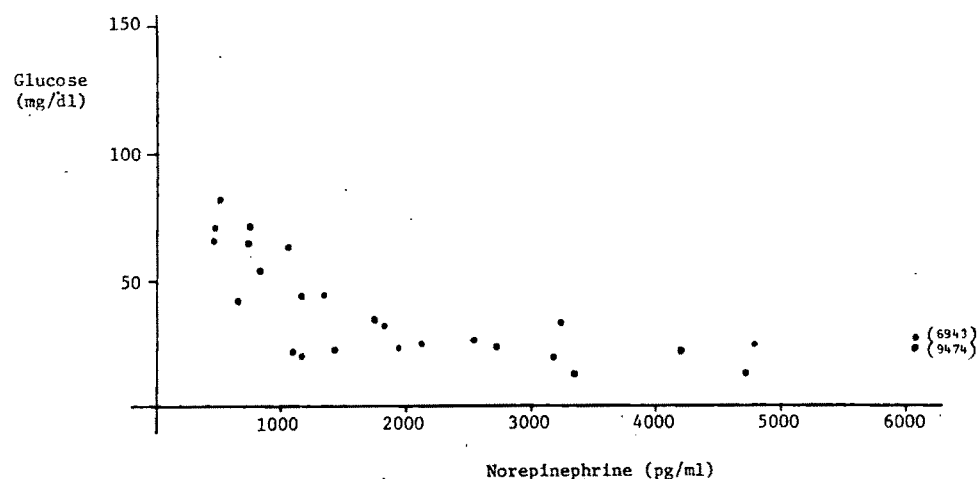


Fig. 4. Fetal plasma glucose levels compared to NE values. Correlation coefficient  $r = -0.56$ ,  $p < 0.01$ .

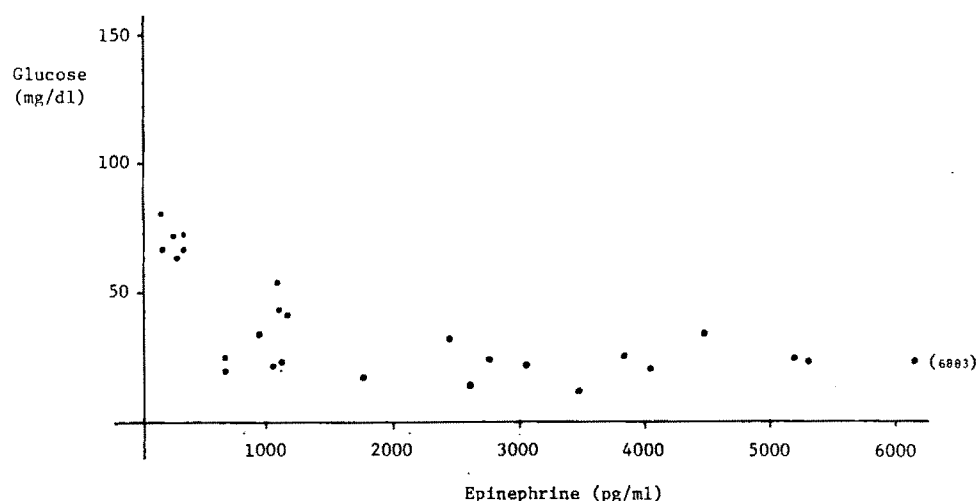


Fig. 5. Fetal plasma glucose levels compared to EPI values. Correlation coefficient  $r = -0.58$ ,  $p < 0.01$ .

rats was  $217.8 \pm 123.3$  pg/ml ( $\pm$  SEM). In response to acute hypoglycemia, there was little change by 1 hour: The EPI level was  $659.3 \pm 183.7$  pg/ml (not significant), whereas by 2 hours the EPI level had increased to  $2,123.5 \pm 536.7$  pg/ml ( $p < 0.05$ ) (Table I). The maternal EPI level remained elevated with a 3-hour value peaking at  $2,548.0 \pm 1,071.2$  pg/ml and then decreasing to  $1,663.8 \pm 420.7$  pg/ml by 4 hours (Fig. 3).

The EPI level in the fetal rat was likewise low in the control animals at  $374.9 \pm 69.8$  pg/ml ( $\pm$  SEM). Similar to the NE value, the fetal EPI level demonstrated a rapid significant elevation by 1 hour to  $1,052.3 \pm 79.3$  pg/ml ( $p < 0.05$ ) (Table II). The fetal EPI level continued to rise with a 2-hour value of  $3,140.8 \pm 499.4$  pg/ml ( $p < 0.01$ ), then peaking at 3 hours to  $4,295.8 \pm$

$538.9$  pg/ml. The fetal EPI value at 4 hours after maternal insulin injection had decreased slightly to  $3,888.3 \pm 340.1$  pg/ml. Fig. 3 reveals that fetal plasma EPI values were higher than those observed in the maternal animals at each interval measured.

The maternal control mean value for plasma dopamine was  $252.4 \pm 85.6$  pg/ml ( $\pm$  SEM). In response to acute hypoglycemia, the maternal dopamine level did not change significantly. The control fetal plasma dopamine value was  $181.9 \pm 39.8$  pg/ml. Similar to the other fetal catecholamine levels, the dopamine level peaked at 3 hours at  $869.0 \pm 203.3$  pg/ml ( $p < 0.05$ ). The physiologic significance or role of this rise in fetal plasma dopamine is unknown at this time.

The fetal NE level (Fig. 4) demonstrated a significant

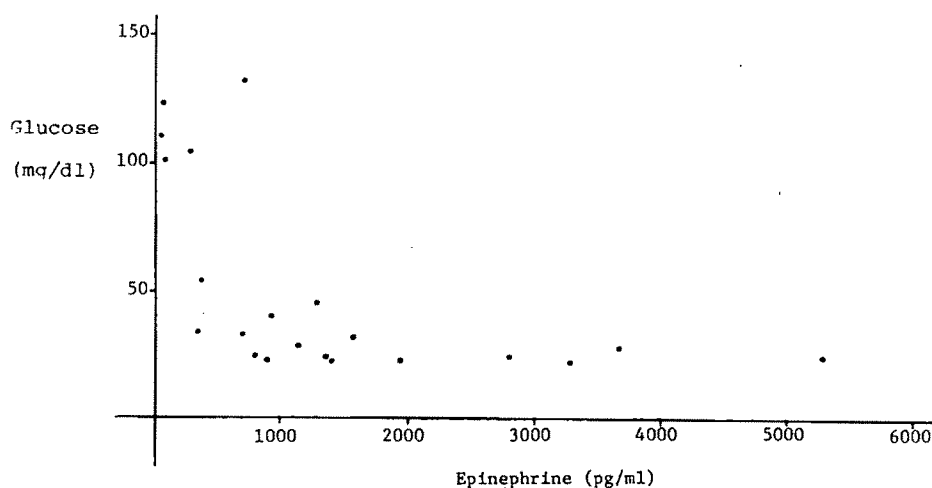


Fig. 6. Maternal plasma glucose levels compared to the EPI values. Correlation coefficient  $r = -0.54$ ,  $p < 0.01$ .

inverse correlation with the plasma glucose value ( $r = -0.56$ ,  $p < 0.01$ ), whereas the maternal plasma NE level was poorly correlated with the maternal plasma glucose level ( $r = -0.36$ , not significant). Figs. 5 and 6 demonstrate the inverse correlation between the plasma epinephrine and plasma glucose levels. A significant correlation was observed in both the maternal ( $r = -0.54$ ,  $p < 0.01$ ) and fetal ( $r = -0.58$ ,  $p < 0.01$ ) rats.

### Comment

The development of chromaffin tissue in the human fetus was first demonstrated by Zuckerkandl in 1901. Since that time anatomic and histochemical studies by multiple authors<sup>21-24</sup> have confirmed that chromaffin tissue in the fetus, the extra-adrenal chromaffin tissue in particular, undergoes extensive development and maturation during fetal life, then degenerates during early childhood. Bioassay and fluorometric assay techniques have been used to demonstrate that from early in fetal life, these tissues contain significant amounts of catecholamines, in particular NE.<sup>21, 23, 24</sup> Ben-Jonathan<sup>25</sup> has demonstrated that plasma EPI, NE, and dopamine levels are higher in the fetal rat than those observed in the adult.

The ability of the fetal chromaffin tissue to respond functionally to stress in utero was first demonstrated by Comline and Silver.<sup>16</sup> These authors measured a rapid output of catecholamines from the adrenal gland in the fetal lamb and calf in response to maternal hypoxia. This response was demonstrable as early as day 80 of gestation in the fetal lamb. Jones and Robinson,<sup>15</sup> in chronically catheterized sheep, found that, in contrast to the predominantly EPI response observed in the ewe

in response to hypoxia, the fetal lamb has a higher plasma catecholamine output of predominantly NE. Artal and co-workers<sup>19</sup> measured the fetal and maternal sheep response to maternal phlebotomy. They described a significant increase in both maternal and fetal catecholamines in response to a 30% loss of maternal blood volume. In their study all three catecholamines increased significantly; however, the greatest rise occurred in fetal NE.

The ability of the human fetus to respond to stress has been evaluated only during the peripartum period. Lagercrantz and associates<sup>17, 26</sup> measured cord blood catecholamine levels and demonstrated that the levels in the normal newborn infant are significantly higher than those in resting adult plasma. Even more important, these authors demonstrated that catecholamine levels obtained from infants born after complicated deliveries, such as breech presentation or hypoxic fetal heart rate patterns, had even higher plasma catecholamine levels. As observed in the animal models, the predominant catecholamine measured in human fetal plasma was NE. In a series of newborn infants, Blouquit and associates<sup>18</sup> also found that the catecholamine levels were significantly higher in infants born after complicated deliveries.

Observations from multiple studies<sup>15, 27, 28</sup> evaluating the transfer of catecholamines across the placenta would suggest that physiologic levels of catecholamines do not significantly cross the placenta. Jones and Robinson<sup>15</sup> with infusion of labeled catecholamines in both the ewe and lamb fetus were unable to demonstrate transfer of active hormone. Saarikoski<sup>27</sup> in an extensive study of <sup>3</sup>H-NE transfer across the human placenta found that, at most, only 10% of labeled hor-



hormone was found in the umbilical vein as active hormone. On the other hand, Saarikoski found that metabolites of NE rapidly appeared in fetal blood. These metabolites were, in fact, higher in fetal plasma than in the maternal circulation. The conclusion drawn from this study was that catecholamines are rapidly metabolized in the placenta by COMT and monoamine oxidase (MAO) to inactive metabolites. Morgan and associates<sup>28</sup> likewise found significant metabolism of labeled catecholamines as they traversed the guinea pig placenta. Catecholamine measurements by Lagercrantz and associates<sup>26</sup> in cord blood revealed higher cord artery levels than umbilical vein levels, again suggesting that the catecholamines measured arose mainly in the fetus. The data in our study demonstrate that the fetal values of all three catecholamines were higher than those observed in the maternal rats. This observation likewise does not support significant maternal-to-fetal diffusion of catecholamines.

Glucose crosses the placenta by facilitated diffusion,<sup>2, 3</sup> thereby resulting in fetal glucose levels that parallel those of the mother; however, they are lower.<sup>2, 4, 14, 29</sup> Episodes of hypoglycemia in the mother have been demonstrated previously to result in an extremely low fetal glucose concentration.<sup>4, 30</sup> This response to insulin-induced maternal hypoglycemia is thought to be directly secondary to inadequate glucose for transport across the placenta, rather than to an insulin effect on the fetus. Insulin, a large polypeptide molecule, does not cross the placenta.<sup>31, 32</sup>

During fetal life the fetus stores surplus fuel substrate in the form of glycogen, protein, and triglycerides.<sup>4-6</sup> Because of the deficiency of the enzymes necessary for utilization of these depot fuels, the fetus in utero appears unable to draw upon these stores during episodes of maternal substrate deficiency.<sup>7-12, 33, 34, 35</sup> Glycogenolysis requires the presence of G-6-PASE and phosphorylase.<sup>8</sup> Girard and associates<sup>7</sup> and others<sup>8-10</sup> have demonstrated that fetal hepatic G-6-PASE and phosphorylase are very low during fetal life, increasing significantly only at term. The absence of fetal gluconeogenesis has also been suggested because of the fetal hepatic deficiency of PEPCK and pyruvate carboxylase.<sup>7, 11, 12, 33, 34, 36</sup> Ballard and Hanson<sup>11</sup> demonstrated low levels of both enzymes in the rat fetus, with gluconeogenesis occurring only postnatally when soluble PEPCK activity rapidly increased.

The gluconeogenic and glycogenolytic enzymes have been experimentally induced by fetal injection of several hormones, including glucagon and catecholamines.<sup>7, 13, 37, 38</sup> Cyclic adenosine monophosphate, the second messenger produced by glucagon and catecholamine activation of adenyl cyclase, has also been shown

to increase gluconeogenic enzyme activity.<sup>37, 39, 40</sup> As mentioned previously, Epstein and associates<sup>14</sup> and Chez and colleagues<sup>41</sup> were unable to measure an elevation of glucagon in response to induced hypoglycemia. Our data, on the other hand, have demonstrated, in the near-term fetal rat, a significant increase in all three catecholamines in response to hypoglycemia. This elevation was most significant for fetal plasma NE. We are aware of no other reports in the English language literature in which the fetal catecholamine response to hypoglycemia was measured.

The type of catecholamine response that we observed in the maternal rat has been previously reported in adult humans<sup>42-44</sup> and rats.<sup>45</sup> Garber and associates<sup>43</sup> demonstrated a fiftyfold rise in EPI and a twofold increase in NE in six adult men 50 minutes after intravenous injection of insulin. In a similar study, Christensen and associates<sup>44</sup> found a strong correlation between plasma glucose concentration and the plasma EPI value in adult men. Pitkanen<sup>45</sup> demonstrated a rapid increase in urinary EPI in response to insulin-induced hypoglycemia in the adult rat.

Baseline and stressed catecholamine concentrations in our study are consistent with those observed in rats<sup>25, 46-49</sup> and other species.<sup>15, 19, 48</sup> We observed control catecholamine levels in our rats similar to those reported by Buhler and associates<sup>48</sup> after anesthetizing the rats with pentobarbital before manipulation. The hypoglycemic animals were handled in a fashion identical to that of the control animals except for the injection of insulin several hours before sampling. Although we are unable to prove that other, nonspecific stress factors did not contribute to the maternal and fetal catecholamine responses observed, the most obvious stress factor appears to be the profound hypoglycemia induced in both the maternal and fetal rats. To minimize the effects of fetal hypoxia during blood sampling, no animal was used in which the maternal cardiorespiratory function had ceased, and only the first half of the litter, i.e., the first five to six fetuses, were sampled from any one rat. We have observed that upon sampling a whole 12-fetus litter, the glucose concentration and catecholamine levels were higher in the last few fetuses. This effect was probably secondary to some degree of hypoxia in these last fetuses.

Goodner and associates,<sup>50</sup> in 1969, made the observation that in the near-term fetal rat stable plasma glucose levels were maintained in spite of maternal insulin-induced hypoglycemia. Our data demonstrated an initial 60% fall in fetal plasma glucose; however, fetal glucose then plateaued in spite of a continued fall in maternal glucose. The etiology of this fetal glucose plateau is unknown. Three possibilities are (1) de-

creased fetal glucose utilization, (2) increased extraction of maternal glucose, and (3) enzyme induction and release of glucose from fetal glucose stores. Although unable to demonstrate a decrease in hepatic glycogen, Goodner and associates<sup>50</sup> suggested that the near-term fetus is, in fact, able to compensate for maternal hypoglycemia with glycogenolysis and possible gluconeogenesis. The observation of Girard and associates<sup>5, 7</sup> of a significant increase in phosphorylase, G-6-PASE, and PEPCK activity within 1 hour after delivery and the beginning of resolution of neonatal hypoglycemia within 2 hours after delivery in the rat would support

the possibility that within 1 to 2 hours the near-term fetus is able to induce the necessary enzymes for release of stored glucose.

In conclusion, these data suggest that in the near-term fetal rat, chromaffin tissue is able to respond to insulin-induced hypoglycemia with a rapid, significant output of all three catecholamines measured. The possibility that the observed plateau of the fetal plasma glucose concentration, in spite of a continued fall in maternal glucose, occurs because of hepatic enzyme induction by the catecholamine elevation can only be speculated at this time.

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# Maternal plasma adrenocorticotropin and cortisol relationships throughout human pregnancy

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Adrenocorticotropin (ACTH) and cortisol in plasma were measured weekly from early in gestation through delivery in five women whose pregnancies were normal. During the twelfth week of pregnancy, the concentration of ACTH in plasma of blood samples obtained between 0800 and 0900 hours was  $23 \pm 4.6$  pg/ml (mean and SEM) and rose progressively to  $59 \pm 16$  pg/ml at 37 weeks. The levels of ACTH in plasma were significantly lower throughout pregnancy than those found in nonpregnant women. During labor and delivery, ACTH levels rose strikingly to values of  $301 \pm 137$  pg/ml. As pregnancy advanced, the concentration of cortisol in plasma increased progressively from  $149 \pm 34$  ng/ml (mean and SEM) at 12 weeks to  $352 \pm 90$  ng/ml at 26 weeks' gestation but changed minimally thereafter until labor commenced, during which values of  $706 \pm 148$  ng/ml were achieved. ACTH and cortisol secretory patterns over a 24-hour period were also investigated in one subject during each trimester of pregnancy. Diurnal variations were observed that were qualitatively similar to those seen in nonpregnant women. From the results of these studies, we conclude that ACTH levels are suppressed in plasma of normal pregnant women but are higher in late pregnancy than in early pregnancy. The rise in plasma ACTH concentrations, as pregnancy advances, in spite of increasing levels of plasma cortisol, estrogen, and progesterone, is suggestive of the possibility that a source of ACTH exists that is not subject to negative feedback control, that the clearance of free cortisol increases as pregnancy advances, or that there is an alteration in the metabolism of the ACTH precursor protein produced by the pituitary and/or placenta. (AM. J. OBSTET. GYNECOL. 139:416, 1981.)

IT HAS BEEN well documented that total serum cortisol concentrations increase during human pregnancy.<sup>1-3</sup> This increase in the level of cortisol is believed to be due primarily to the threefold increase in

corticosteroid-binding globulin (CBG) levels that accompanies pregnancy.<sup>4</sup> On the other hand, the measurement of free or unbound cortisol in plasma of pregnant women has given rise to results which are conflicting. In early studies, Booth and associates<sup>5</sup> and Murray<sup>6</sup> did not detect an appreciable increase in the levels of unbound cortisol in plasma during normal pregnancy. However, in more recent studies it was found that the levels of free cortisol in plasma are higher in pregnant than those in nonpregnant women.<sup>7, 8</sup>

There have been no reports of results of studies in which plasma adrenocorticotropin (ACTH) levels were quantified in the same women throughout pregnancy. Most investigators have been concerned with ACTH levels in umbilical cord plasma of newborn infants and in plasma obtained from women during labor or delivery. It has been demonstrated that plasma ACTH and

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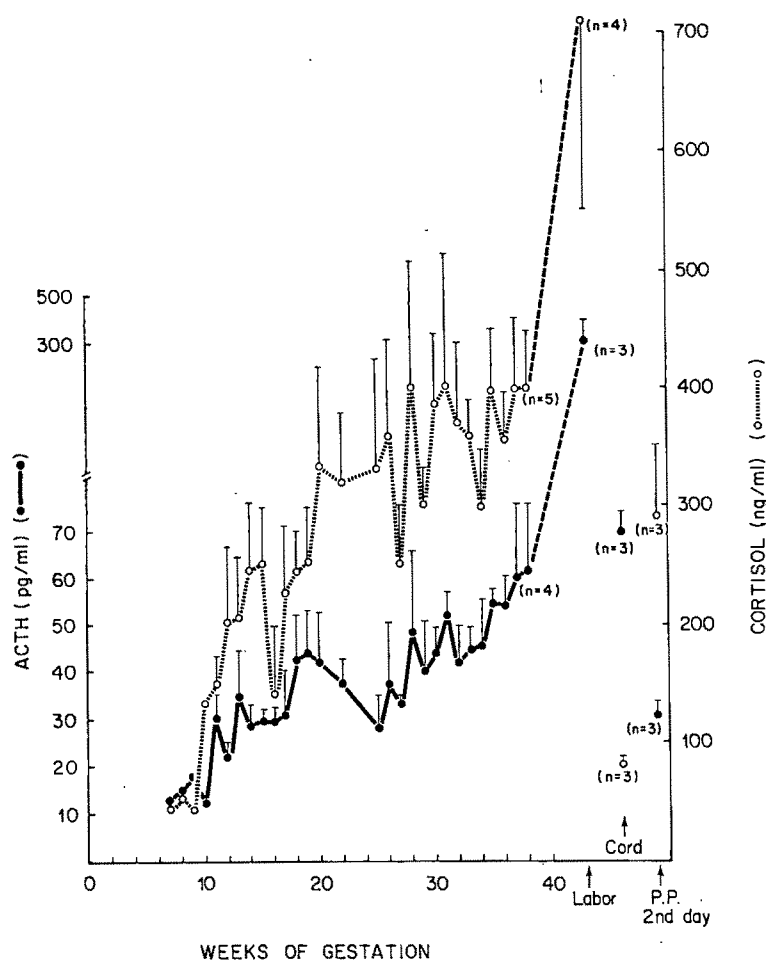


**Fig. 1.** Plasma concentrations of ACTH and cortisol during normal pregnancy. Blood samples were obtained weekly at 0800 to 0900 hours from five normal pregnant women and from three women during labor and on the second postpartum day. In addition, umbilical cord plasma was obtained from the newborn infants of three of these subjects. The mean plasma concentrations for ACTH (picograms per milliliter) are denoted by solid circles whereas plasma cortisol levels (nanograms per milliliter) are denoted by open circles. The vertical bars correspond to the magnitude of the standard error of the mean.

cortisol concentrations are significantly higher in pregnant women during labor than those in normal, resting, nonpregnant individuals.<sup>9-13</sup>

It has been established that episodic secretions of ACTH and cortisol occur in nonpregnant subjects.<sup>9, 14, 15</sup> There have been no reports of results of studies of secretory patterns of ACTH and cortisol during the course of human gestation.

The purpose of the present investigation was to evaluate pituitary and adrenal function throughout pregnancy by measuring plasma ACTH and cortisol in a prospective study in which each woman served as her own control. In addition, the relationship of ACTH and cortisol secretory activity during the day at various stages of gestation was evaluated.

#### Material and methods

ACTH and cortisol were quantified in plasma of five pregnant women beginning at the seventh to eleventh gestational week until delivery. The ages of the women ranged from 17 to 28 years. The pregnancies of these five women were normal.

While the subjects were resting in the lateral recumbent position, blood samples were obtained at weekly intervals between 0800 and 0900 hours after an overnight fast. In addition, blood samples were obtained from some subjects during labor and delivery and 2 days post partum. Mixed umbilical cord blood was obtained from some of the newborn infants of these women.

The 24-hour secretory patterns of ACTH and cor-

**Table I.** Plasma ACTH levels during early and late pregnancy

| Subject | ACTH (pg/ml) |      |       |             |       |        | p Value |
|---------|--------------|------|-------|-------------|-------|--------|---------|
|         | 9-14 Weeks   |      |       | 35-40 Weeks |       |        |         |
|         | Mean         | SEM  | Range | Mean        | SEM   | Range  |         |
| A       | 30           | ±3.5 | 25-39 | 55          | ±4.0  | 48-62  | <0.01   |
| B       | 15           | ±1.7 | 12-18 | 34          | ±6.5  | 21-47  | <0.05   |
| C       | 43           | ±3.3 | 38-52 | 83          | ±12.0 | 59-108 | <0.05   |
| D       | 28           | ±2.3 | 23-33 | 50          | ±4.2  | 39-59  | <0.01   |

**Table II.** Plasma cortisol levels during early and late pregnancy

| Subject | Cortisol (ng/ml) |     |         |             |     |         | p Value |
|---------|------------------|-----|---------|-------------|-----|---------|---------|
|         | 9-14 Weeks       |     |         | 35-40 Weeks |     |         |         |
|         | Mean             | SEM | Range   | Mean        | SEM | Range   |         |
| A       | 313              | ±50 | 220-460 | 336         | ±13 | 300-360 | NS      |
| B       | 77               | ±20 | 40-130  | 314         | ±22 | 260-390 | <0.001  |
| C       | 165              | ±36 | 120-210 | 430         | ±11 | 410-460 | <0.001  |
| D       | 270              | ±50 | 180-410 | 557         | ±42 | 490-680 | <0.01   |
| E       | 333              | ±22 | 270-400 | 823         | ±51 | 720-880 | <0.001  |

NS: Not significant.

tisol in plasma were studied during each trimester in one normal pregnant woman, Subject F. This woman was admitted to the Clinical Research Center at the University of Texas Health Science Center at Dallas for a 2-day period during each trimester of pregnancy. She was given a general diet but her activities were restricted to the study room for a 48-hour period. The first night of each study served as a stabilization time. On the second day an 18-gauge Longdwell catheter with a three-way stopcock and heparin lock syringe was inserted into an arm vein for blood sampling. In each sampling period 3 ml of blood was drawn and the number of sampling intervals was designed to keep the total amount of blood withdrawn at a minimum. Blood samples were obtained with limited disturbance of the test subject in the following time periods: every 2 hours from 0800 to 0400; every 5 minutes between the time intervals 0400 and 0430, 0500 and 0530, and 0630 and 0800. This experimental design was chosen in the hope that blood sampling would be conducted during the highest peak in ACTH secretory activity, as described by Gallagher and associates.<sup>15</sup> The sleep and awake periods of the subject were recorded.

Blood samples for ACTH measurement were collected in tubes containing ethylenediaminetetraacetate (EDTA) (1 mg/ml) and stored at 4° C for no more than 60 hours before centrifugation at 1° to 2° C. The plasma was removed by aspiration and stored at -20° C

until assayed. The stability of ACTH at 4° C in plasma containing EDTA has been established previously<sup>12</sup> and confirmed in the present investigation with the use of blood obtained at 1000 hours from two normal men. ACTH was quantified by radioimmunoassay (RIA) according to the procedure of Oliver<sup>16</sup> and Vague and associates,<sup>17</sup> as modified by Winters and associates.<sup>12</sup> The antiserum was developed in rabbits against porcine ACTH<sup>17</sup> and was a gift from Dr. C. Oliver (Marseille, France). The specificity of this antiserum has been investigated extensively in cross-reactivity studies with the use of peptides closely related to melanocyte-stimulating hormone (MSH) and ACTH.<sup>18</sup> Compared to those of porcine ACTH, the antibodies in this antiserum recognized with equal affinity the following peptides: ACTH 1-14, ACTH 1-16, and ACTH 11-24. The following peptides are not recognized: ACTH 4-10, ACTH 1-10, ACTH 25-39,  $\alpha$ -MSH, and  $\beta$ -MSH. Hence, it is reasonable to assume that the antigenic determinants on the ACTH molecule recognized by antibodies in this antiserum lie within that portion of ACTH equivalent to ACTH 11-16. For a reference standard, we employed  $\alpha$ -ACTH purified from human pituitary glands, which was a gift from Dr. C. H. Li (San Francisco, California). The interassay variation of the ACTH RIA was 13%. All the plasma values for ACTH in this report were determined in a single assay. Plasma cortisol was quantified by a direct RIA de-



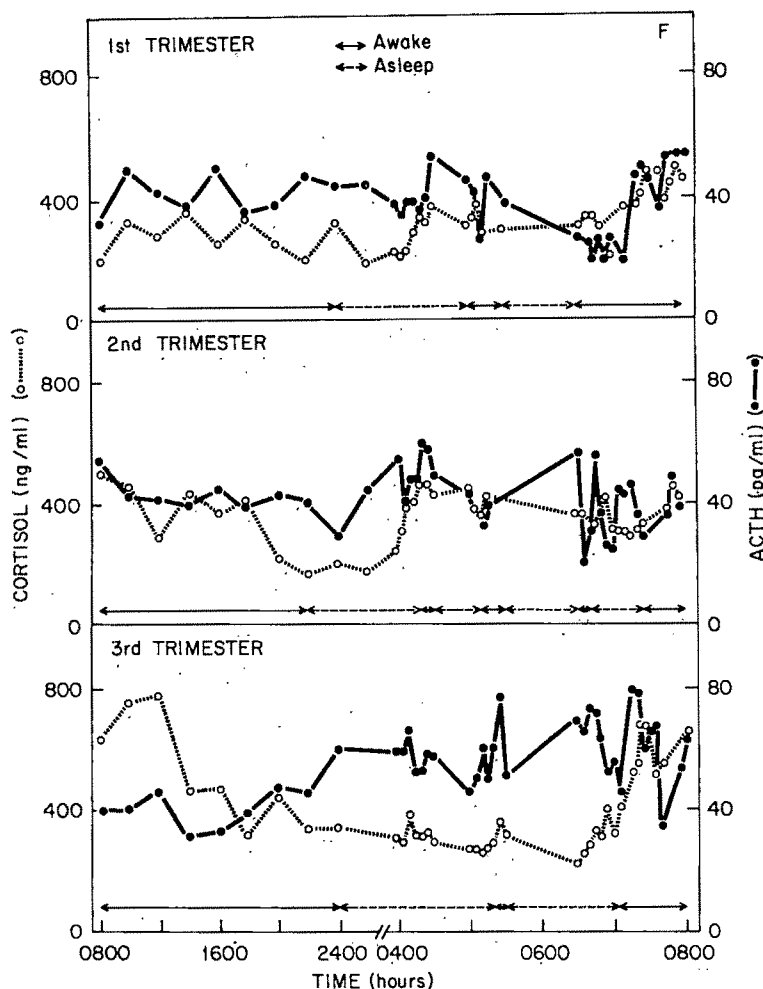


Fig. 2. The 24-hour ACTH and cortisol plasma levels in a normal pregnant woman (Subject F) during each trimester. Blood samples were collected through an indwelling catheter, as described in Material and methods. Plasma ACTH levels are denoted by solid circles and plasma cortisol is indicated by the open circles. Sleep and awake periods are also depicted.

scribed by Gomez-Sanchez and associates.<sup>10</sup> The interassay variation was 14% and the intra-assay variation was 7% for the cortisol RIA.

## Results

**Plasma ACTH.** The concentrations of ACTH in plasma (Fig. 1) in four women with normal pregnancies increased from  $23 \pm 4.6$  pg/ml (mean and SEM) during the twelfth week of gestation to  $59 \pm 16$  pg/ml at 37 weeks. (Blood samples from the fifth woman were collected improperly for ACTH determination and were not included in this study.) During labor and delivery, plasma ACTH levels increased strikingly to  $301 \pm 137$  pg/ml while umbilical cord plasma ACTH levels were  $69 \pm 18$  pg/ml. After delivery, the maternal plasma ACTH had decreased to  $30.3 \pm 8$  pg/ml on the second

postpartum day. The values for ACTH levels in plasma obtained during early and late gestation for each subject are given in Table I. When these data were analyzed by the paired t test, it was found that the concentration of ACTH in plasma during late gestation differed significantly from that of early gestation.

**Plasma cortisol.** By 11 weeks of gestation the plasma cortisol level was  $149 \pm 34$  ng/ml in five normal pregnant subjects. As illustrated in Fig. 1, plasma concentrations of cortisol in these subjects rose progressively throughout the second trimester and attained a level of  $352 \pm 90$  ng/ml (mean and SEM) during the twenty-sixth week of gestation. Thereafter, the plasma concentration remained elevated but changed little until labor and delivery. During labor and at the time of delivery, plasma cortisol concentrations rose to  $706 \pm 148$

ng/ml. By the second postpartum day, the maternal cortisol levels had fallen to  $296 \pm 90$  ng/ml. The plasma cortisol values obtained during early and late gestation for the individual subjects are given in Table II. When these data were analyzed by the paired *t* test, it was found that the concentration of cortisol in plasma during late gestation differed significantly from that of early gestation in four of five subjects. The cortisol level in umbilical cord plasma was  $83 \pm 25$  ng/ml.

**Secretory activity of ACTH and cortisol during pregnancy.** The secretory patterns of plasma ACTH and cortisol during each trimester of pregnancy of Subject F are depicted in Fig. 2. A diurnal rhythm of plasma cortisol is evident during each trimester with highest levels detected at 0700 to 0900 hours and lowest levels found during the early evening and during sleep periods. The difference between morning and evening concentrations of cortisol in plasma appears to increase with advancing gestation. A definite diurnal rhythm of plasma ACTH is less obvious but becomes more evident during the third trimester. Secretory spikes of ACTH were often, but not always, followed by increases in plasma cortisol.

#### Comment

In the present study of normal pregnant women, we found a progressive rise in total plasma cortisol levels with advancing gestation, a finding which is in agreement with the results of other investigators.<sup>1-3</sup> This increase in plasma cortisol levels is believed to be secondary to a rise in circulating levels of CBG.<sup>4</sup> Some investigators<sup>7, 8, 20</sup> have found an increase in free cortisol in plasma during pregnancy, whereas others have found levels of free cortisol which do not differ from those in nonpregnant persons.<sup>5, 6</sup> Thus, it remains somewhat unclear whether free cortisol is increased in plasma during pregnancy.

The levels of plasma cortisol rose dramatically during labor and delivery, presumably the result of stress. In the few samples of umbilical cord plasma studied, we found, as have others,<sup>9, 21</sup> that the levels of cortisol were lower than those in maternal plasma. Krauer<sup>22</sup> found no correlation between maternal and cord plasma cortisol concentrations and suggested, as did Dormer and France,<sup>21</sup> that the cortisol found in fetal blood is produced in the fetus.

There are only a few reports concerning the levels of ACTH in maternal plasma during human pregnancy. Allen and associates<sup>13</sup> detected a progressive rise in ACTH levels during pregnancy in a woman with Nelson syndrome. Genazzani and associates<sup>23</sup> reported that the levels of immunoreactive ACTH in plasma of pregnant women were above the range of values found

in nonpregnant subjects but that ACTH levels decreased near term. A progressive rise of plasma ACTH levels with advancing gestation (but within the range of plasma ACTH levels obtained for nonpregnant subjects) was reported by Rees and associates.<sup>20</sup> Mukherjee and Swyer<sup>24</sup> studied ACTH levels in plasma of normal pregnant women at different gestational ages. They observed that the concentration of ACTH in plasma was lower in women during the third trimester of pregnancy than in nonpregnant women.

The experimental design of the present investigation differed from those of previous studies in that each woman in the present study served as her own control throughout pregnancy. Our results are suggestive that there is a rise in the level of ACTH in plasma as well as in total plasma cortisol from early gestation until term. However, the ACTH levels in plasma of the pregnant subjects throughout gestation ranged from 12 to 60 pg/ml (Fig. 1), values which are considerably less than those found in a group of normal ovulatory women, in whom plasma ACTH levels ranged from 78 to 120 pg/ml.<sup>25</sup> In the latter study, we found significantly lower levels of ACTH in plasma in a group of women treated with oral contraceptives compared to the levels of ACTH in plasma of ovulatory subjects. In addition, it has been demonstrated that ACTH secretion by pituitary cells in culture can be suppressed by adding progesterone to the culture medium.<sup>26</sup> Taken together, these findings are suggestive of a direct suppression of ACTH by estrogens and/or progestogens.

It is more difficult to explain the rise in ACTH levels in plasma as pregnancy advanced. High levels of ACTH have been detected in placental extracts.<sup>20, 23</sup> Thus, it is possible that if ACTH were produced by the placenta and secreted into the maternal compartment, a progressive rise in ACTH during gestation could be explained. Presumably placental ACTH secretion would not be subject to negative feedback control by cortisol. Another explanation for the increasing levels of ACTH during pregnancy may be increased clearance of free cortisol in plasma, e.g., by renal excretion, since there is an increase in the glomerular filtration rate with advancing gestation.<sup>27</sup> An increased clearance of free cortisol in plasma might lead to an increase in ACTH secretion. Another possible explanation for the rise in ACTH levels in plasma as pregnancy advances might be an alteration in the metabolism of the 31 K protein, the presumed prohormone of ACTH and other pituitary and neural peptides.<sup>28</sup> In a recent report it was suggested that other metabolites of the 31 K protein are altered during pregnancy.<sup>29</sup>

Numerous investigators have measured ACTH in maternal plasma during labor and in umbilical cord



plasma at delivery. The findings of this study of an increase in maternal ACTH during labor and delivery are in agreement with the findings of others.<sup>9-13</sup> We found that umbilical cord plasma ACTH levels were lower than those in maternal plasma at the time of labor and delivery; these findings are in agreement with those of our previous studies<sup>12</sup> and with those of others.<sup>10</sup> On the other hand, some investigators<sup>13, 24</sup> have reported the finding of no significant difference in ACTH levels in cord plasma compared to levels in plasma of normal pregnant women at delivery.

The 24-hour secretory patterns of ACTH and cortisol have been investigated in nonpregnant subjects.<sup>9, 14, 15</sup> The results of our studies on Subject F (Fig. 2) were qualitatively similar to those reported in studies of nonpregnant subjects. A definite diurnal rhythm was detected for cortisol in plasma during each trimester, with a trend toward a greater morning-evening difference in cortisol levels as pregnancy progressed. A diurnal rhythm for ACTH was less apparent. Berson and Yalow<sup>9</sup> found that a well-marked diurnal rhythm of ACTH was not always evident in their healthy control subjects.

The intensity and variation in the levels of ACTH in plasma in the pregnant women of this study were more marked than those of cortisol. The peaks in ACTH levels persisted for 5 to 15 minutes and might not have been observed if less frequent sampling intervals had been employed. In most instances, increased ACTH

levels in plasma preceded in time or accompanied a more gradual and sustained elevation of cortisol. These findings are in agreement with those of previous investigators<sup>9, 15</sup> in that the secretory patterns of ACTH remained unaltered by the duration of pregnancy. We also found that augmented ACTH secretion could commence even while cortisol levels were elevated. We suggest, as did Gallagher and associates,<sup>15</sup> that the cortisol feedback mechanism controlling ACTH secretion is probably not a one-to-one relationship and that the central nervous system-hypothalamus are centers of control of the episodic secretory activity of ACTH release.

Based on the findings of the present study, we conclude that maternal plasma levels of ACTH are suppressed during normal pregnancy but that a progressive rise of ACTH occurs throughout pregnancy, despite increasing levels of plasma cortisol, estrogen, and progesterone.

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# The glucose tolerance test as a means of identifying intrauterine growth retardation

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The purpose of this prospective, nonconcurrent study was to determine whether maternal hypoglycemia, as measured by a 3-hour glucose tolerance test, is associated with pregnancies resulting in growth-retarded infants. Our results demonstrate that maternal hypoglycemia is significantly associated with intrauterine growth retardation other than the low birth weight type and that the 3-hour glucose tolerance test is an effective early screening test in detecting pregnancies at risk of fetal growth retardation. (AM. J. OBSTET. GYNECOL. 139:423, 1981.)

INTRAUTERINE GROWTH RETARDATION (IUGR) remains one of the major perinatal problems, carrying a perinatal mortality rate three times higher than that in the total population,<sup>1</sup> with the survivors displaying a high incidence of neurological and intellectual deficiencies.<sup>2, 3</sup>

Traditionally, IUGR has been defined based on weight criteria, such as term birth weight of less than 2,500 gm or birth weight less than the third or tenth percentile for gestational age.<sup>4, 5</sup> On the other hand, Holmes and colleagues<sup>6</sup> have distinguished two important but different growth retardation patterns not based on birth weight criteria: (1) a ponderal index (PI) less than the third percentile and (2) crown-heel length (CHL) less than the one and one-half percentile. Such patterns of growth retardation have recently been referred to as non-low birth weight IUGR (non-LBW IUGR).<sup>7</sup> Miller<sup>8</sup> advocated assessment of IUGR by these indices because low PI has been associated with an increased mortality risk and because up to 50% of low PI infants had weights that were adequate for gestational age; thus, the diagnosis would have been missed if birth weight criteria were applied.<sup>9</sup> Routine

diagnostic indices, such as poor fundal growth, poor maternal weight gain, and low estriol secretion, while indicative of LBW-IUGR outcome, have not proved predictive of non-LBW-IUGR outcome.<sup>7</sup>

Although the etiology of IUGR remains obscure, multiple maternal factors affecting the incidence of fetal growth retardation are known.<sup>9-12</sup> However, Low and Galbraith<sup>1</sup> and others could not demonstrate any cause in half of their patients. Maternal malnutrition has frequently been cited as an explanation for that large proportion where the cause is unknown.<sup>13, 14</sup>

Abell and Beischer<sup>15, 16</sup> found the mean 3-hour blood glucose level following an oral glucose tolerance test (GTT) to be significantly lower in LBW IUGR and also reported an association between maternal hypoglycemia, defined as any value on a GTT at or below the fifth percentile, and an increased incidence of LBW IUGR. These findings suggest that alterations in carbohydrate metabolism may be associated with growth retardation. The purpose of this prospective, nonconcurrent study was to repeat Abell and Beischer's study and to test the usefulness of the GTT as a clinical tool in predicting LBW and non-LBW IUGR outcome.

## Material and methods

All deliveries at The Johns Hopkins Hospital occurring between February, 1978, and June, 1978, were prospectively coded, and all patients who had a GTT were included in the study, except for those patients diagnosed as having gestational diabetes mellitus. The GTTs were performed to screen for diabetes mellitus among high-risk patients (previous large baby, obese

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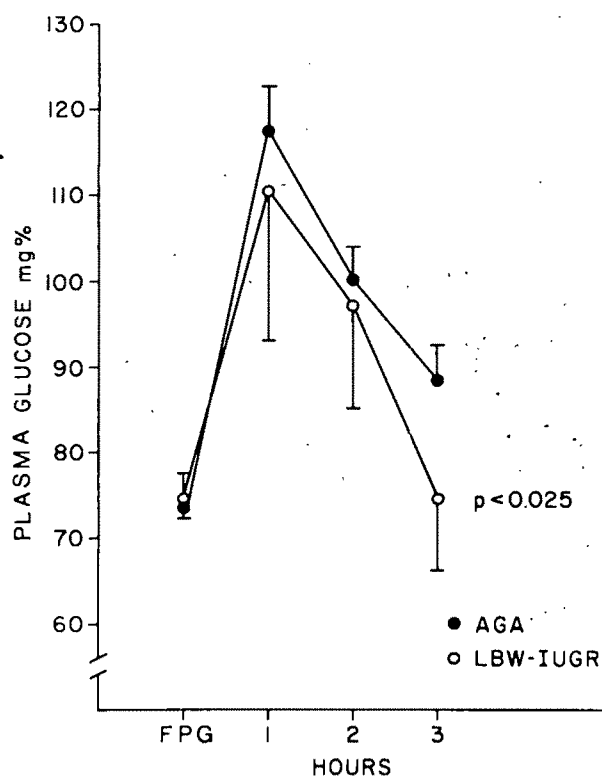


Fig. 1. Comparison of plasma glucose values (mean  $\pm$  2 SEM) during a 3-hour GTT in AGA and LBW IUGR. FPG = Fasting plasma glucose.

mothers, and positive family history for diabetes mellitus). The study population consisted of 122 patients: 100 patients whose pregnancies resulted in infants with normal growth characteristics for their gestational ages (AGA) and 22 patients whose pregnancies resulted in IUGR defined by either a low birth weight (LBW IUGR,  $N = 10$ ) or an adequate birth weight with a low PI (non-LBW IUGR,  $N = 12$ ).

Patients were instructed to continue their normal diet and then fast from midnight the night before the test. Venous blood was collected in fluorinated tubes. Assays of plasma glucose were performed by the glucose oxidase method. A fasting plasma glucose specimen was obtained, then a standard 100 gm oral GTT was performed, with plasma glucose measured in hourly blood specimens for 3 hours after the ingestion of glucose.

The gestational age was determined by the best obstetric estimate and confirmed by ultrasonic examinations and Dubowitz scores.

Student's *t* test and the chi-square test with the Yates corrections were used for statistical analyses.

## Results

The AGA, LBW IUGR, and non-LBW IUGR groups were compared for various maternal risk factors known to be associated with increased incidences of

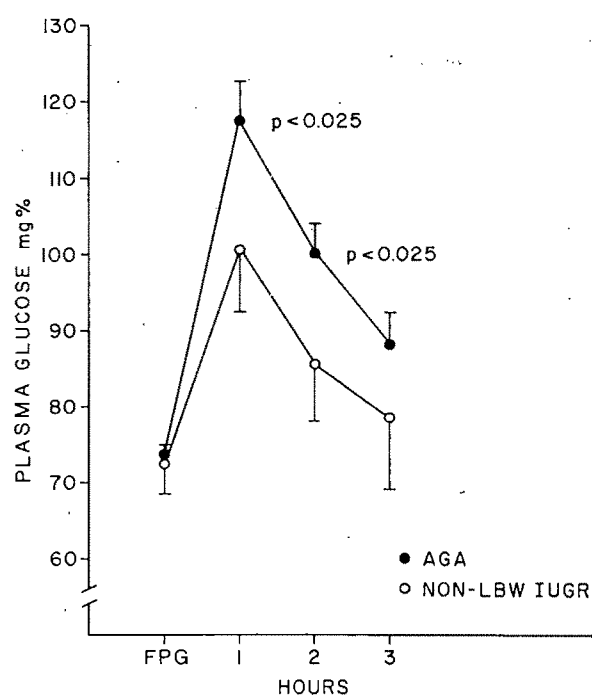


Fig. 2. Comparison of plasma glucose values (mean  $\pm$  2 SEM) during a 3-hour GTT in AGA and non-LBW IUGR. FPG = Fasting plasma glucose.

IUGR<sup>9-12</sup> and no statistical differences were found (Table I).

As the GTTs were not performed at the same gestational age in all AGA patients, the mean fasting, 1-, 2-, and 3-hour glucose values at 26 to 27, 28 to 32, 33 to 36, and 37 to 40 weeks were compared. The mean fasting plasma glucose was significantly lower in the group at 37 to 40 weeks, but no significant difference was found in the other three gestational groups and the 1-, 2-, and 3-hour mean values were not significantly different, irrespective of gestational age.

When the mean plasma glucose level was compared in AGA and LBW IUGR, there was no difference in the mean fasting values, and, although there was a tendency to lower values at the 1- and 2-hour readings, it was not significant except at the 3-hour reading (Fig. 1). When the mean plasma glucose level was compared in AGA and non-LBW IUGR, there was no difference in the mean fasting values; however, the difference was statistically different at the 1- and 2-hour readings but fell short of achieving statistical significance at the 3-hour reading (Fig. 2).

The plasma glucose percentiles for our normal population were determined (Table II) and hypoglycemia was defined as any value of the GTT at or below the fifth percentile. The overall incidence of hypoglycemia in the AGA group was 16%, and there was an apparent increasing trend with increasing gestational age. However, this was not statistically significant.



Table I. Maternal epidemiologic factors

|                                     | Normal (N = 100) | LBW IUGR (N = 10) | Non-LBW IUGR (N = 12) |
|-------------------------------------|------------------|-------------------|-----------------------|
| Medical assistance (%)              | 87               | 90                | 83                    |
| Age (years)                         | 21.26 ± 4.71     | 21.21 ± 4.8       | 20.86 ± 4.9           |
| Negro (%)                           | 70               | 80                | 66                    |
| Nonpregnant weight (pounds)         | 142.38 ± 37.61   | 141.59 ± 35.64    | 140.89 ± 36.24        |
| Weight gain (pounds)                | 22.70 ± 9.51     | 22.9 ± 9.89       | 23.36 ± 10.16         |
| Weeks under care                    | 24.11 ± 6.38     | 24.86 ± 3.46      | 24.98 ± 3.67          |
| Anemia (hematocrit = <30%) (%)      | 12               | 20                | 25                    |
| Cigarette smoking (>10/day) (%)     | 14               | 20                | 16                    |
| Ethanol intake (>1 ounce/day) (%)   | 19               | 10                | —                     |
| Intake of opioids (%)               | 10               | 10                | —                     |
| Prior IUGR (%)                      | 2                | —                 | —                     |
| Hypertensive disorders (%)          | 9                | 10                | 8                     |
| Gestational age at delivery (weeks) | 37.28 ± 1.28     | 37.63 ± 1.95      | 37.44 ± 1.88          |

Table II. Plasma glucose values

|        | Fifth percentile (mg/100 ml) | Fiftieth percentile (mg/100 ml) | Ninety-fifth percentile (mg/100 ml) |
|--------|------------------------------|---------------------------------|-------------------------------------|
| FPG    | 65                           | 74                              | 86                                  |
| 1-Hour | 75                           | 122                             | 155                                 |
| 2-Hour | 67                           | 104                             | 139                                 |
| 3-Hour | 57                           | 87                              | 122                                 |

FPG: Fasting plasma glucose.

Table III. Incidence of hypoglycemia in AGA, LBW IUGR, and non-LBW IUGR

| GTT obtained at                              | AGA          | LBW IUGR   | Non-LBW IUGR |
|----------------------------------------------|--------------|------------|--------------|
| All gestational ages (26-40 weeks) (N = 122) | 16/100 (16%) | 2/10 (20%) | 5/12 (42%)   |
| 28-32 weeks (N = 71)                         | 6/55 (11%)   | 1/7 (14%)  | 5/9 (55%)*   |

\*p < 0.01.

The incidence of hypoglycemia in AGA, LBW IUGR, and non-LBW IUGR is presented in Table III. No difference was found between the AGA and LBW IUGR groups and, although there was a 2½-fold increase in the non-LBW IUGR group, it fell short of achieving statistical significance. When only those patients who underwent the GTT at 28 to 32 weeks of gestation were considered, no difference could be found between the AGA and LBW IUGR groups, but the increased incidence of hypoglycemia in the non-LBW IUGR group was statistically significant.

In order to assess the value of the GTT as a clinical tool in predicting the LBW and non-LBW IUGR outcome, the sensitivity, specificity, positive, and negative predictive values of hypoglycemia were determined for the LBW and non-LBW IUGR groups (Tables IV and V). In the absence of hypoglycemia, there is a 90% probability that the pregnancy will not result in either

Table IV. Evaluation of the GTT as a screening test in predicting LBW IUGR

|               | LBW IUGR | AGA |
|---------------|----------|-----|
| Hypoglycemia  | 1        | 6   |
| Normoglycemia | 6        | 49  |
| Total         | 7        | 55  |

Sensitivity, 14.2%; specificity, 89.1%; positive predictive value, 14.2%; negative predictive value, 89.2%.

Table V. Evaluation of the GTT as a screening test in predicting non-LBW IUGR

|               | Non-LBW IUGR | AGA |
|---------------|--------------|-----|
| Hypoglycemia  | 5            | 6   |
| Normoglycemia | 4            | 49  |
| Total         | 9            | 55  |

Sensitivity, 55.5%; specificity, 89.1%; positive predictive value, 45.4%; negative predictive value, 92.4%.

form of IUGR. However, in the presence of hypoglycemia, only 15% will result in LBW-IUGR but approximately 50% will result in non-LBW IUGR. The sensitivity is around 15% for LBW IUGR but increases to 55% for non-LBW IUGR.

Comment

Insulin is believed to be the fetal growth hormone<sup>17</sup> and it is partly regulated by fetal glucose levels<sup>18</sup> that are, in turn, determined by the glucose gradient between the maternal and fetal compartments.<sup>19</sup> Thus, maternal hypoglycemia would lead to fetal hypoglycemia and hypoinsulinemia and possibly to growth retardation. One might postulate that LBW IUGR and non-LBW IUGR are different expressions of the same disease process, the former being the more severe and the latter the milder expressions of the disease. Thus, the more severe the hypoglycemia, then the greater the

likelihood of LBW IUGR. Abell and Beischer<sup>20</sup> reported that with increasing numbers of hypoglycemic values on the GTT, there is an increasing incidence of LBW IUGR. Although the mean 3-hour glucose value was significantly lower in LBW IUGR compared to AGA, the incidence of hypoglycemia in LBW IUGR was not significantly increased.

Abell,<sup>21</sup> in a study of 5,000 consecutive patients who underwent a GTT in the third trimester of pregnancy, found an incidence of 12.8% LBW IUGR when one hypoglycemic value was present and an incidence of 18.4% when more than one hypoglycemic value was present. These incidences were significantly greater than the 7.2% incidence of LBW IUGR in the presence of normoglycemia. This is in agreement with our finding of a 15% incidence of LBW IUGR in the presence of hypoglycemia. Study group size and selection factors in our study may explain why the association of hypoglycemia and LBW IUGR was not found to be statistically significant. Our pregnant patients were at risk for gestational diabetes mellitus and, although the GTTs excluded patients with gestational diabetes mellitus, we cannot exclude the possibility that our study group tended to have larger babies compared to those of pregnant women not at risk to develop gestational diabetes mellitus.

Another question that needs to be answered is what causes the maternal hypoglycemia. Maintenance of a normal glucose tolerance in pregnancy depends on the interaction of maternal factors, such as insulin and insulin receptors, glucagon, cortisol, and prolactin, with placental factors, particularly human placental lactogen, estrogen, and progesterone. Hypoglycemia is the result of a relative hyperinsulinemic state that can be due to either increased insulin levels or insulin receptors or to a decrease of one or more of the diabetogenic hormones. We are currently conducting a study to examine these factors in an effort to determine the cause(s) of maternal hypoglycemia in IUGR.

In summary, maternal hypoglycemia was found to be significantly associated with non-LBW IUGR. An association with LBW IUGR could not be demonstrated, possibly because of the small size of the study population. The GTT at 28 to 32 weeks appears to be an early and effective screening test for non-LBW IUGR. Further studies are needed to determine the cause(s) of hypoglycemia in association with IUGR, since it could have relevance to the cause of IUGR.

We wish to thank Mrs. Patricia Murphy for her valuable guidance in the manuscript preparation.

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# Lack of relationship between prolactin and adrenal steroidogenesis in the ovine fetus

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The role of fetal prolactin (PRL) as a fetal adrenotropic hormone was investigated in eight chronically catheterized fetal lambs. Catheters were placed for measurement of plasma prolactin (PRL), cortisol (F), dehydroepiandrosterone (DHEA), dehydroepiandrosterone sulfate (DHEA-S), and androstenedione (A). Daily (9 AM) samples were obtained until the onset of labor. In four animals 2-Br- $\alpha$ -ergocryptine (BC) was administered subcutaneously (1 mg/12 hours) for 11 to 21 days prior to delivery. Four animals served as controls. No difference between groups was noted in the duration of gestation. PRL increased in the control animals from  $8.4 \pm 2.4$  to  $43.0 \pm 5.6$  ng/ml (mean  $\pm$  SEM) at the time of delivery whereas in animals that received BC it decreased within 24 hour of administration and remained below 3 ng/ml throughout the study period. No differences were noted in the concentration of F, DHEA, DHEA-S, and A between groups. These results suggest that PRL does not play a role in adrenal steroidogenesis or in the initiation of parturition. (AM. J. OBSTET. GYNECOL. 139:427, 1981.)

THE STUDIES OF Liggins and associates<sup>1</sup> have established that the fetal hypothalamic-pituitary-adrenal axis plays a primary role in the initiation of parturition in sheep. Several days prior to delivery the physiologic interplay of these organs results in an increase in fetal plasma cortisol (F) concentration. This event is a prerequisite to the onset of normal labor. Since the infusion of adrenocorticotrophic hormone (ACTH) into normal or hypophysectomized lamb fetuses prematurely induces labor, fetal ACTH has been thought to be the adrenotropic hormone which stimulates this rise in F. However, attempts to document an increase in fetal plasma ACTH prior to the increase in F have been unsuccessful.<sup>2, 3</sup> Also it has been shown that the administration of ACTH to an intact fetus will result in the initiation of labor sooner than when ACTH is adminis-

tered to a hypophysectomized fetus.<sup>4</sup> These observations have suggested that pituitary hormones other than ACTH may be responsible, directly or indirectly, for the stimulation of the fetal adrenal gland.

Recently, several studies in humans suggested that prolactin (PRL) may be an adrenotropic hormone. Correlations between fetal adrenal weight and PRL concentrations indicate that fetal PRL may play a role in the growth of the human fetal adrenal gland.<sup>5</sup> Furthermore, there appears to be a better correlation between growth of the fetal adrenal zone with changes in the fetal PRL concentrations than with concentrations of fetal ACTH.<sup>5, 6</sup> Also a recent study indicates that PRL may affect adrenocortical androgens in human adults.<sup>7</sup>

In sheep, both fetal plasma PRL and F increase at a similar rate as pregnancy advances.<sup>8</sup> For these reasons we hypothesized that in sheep the fetal PRL might be involved in fetal adrenal steroidogenesis. To investigate this hypothesis, in late pregnancy we measured daily concentrations of fetal F, dehydroepiandrosterone (DHEA), dehydroepiandrosterone sulfate (DHEA-S), and androstenedione (A) in normal fetuses and in fetuses made hypoprolactinemic by the administration of 2-bromo- $\alpha$ -ergocryptine (BC).

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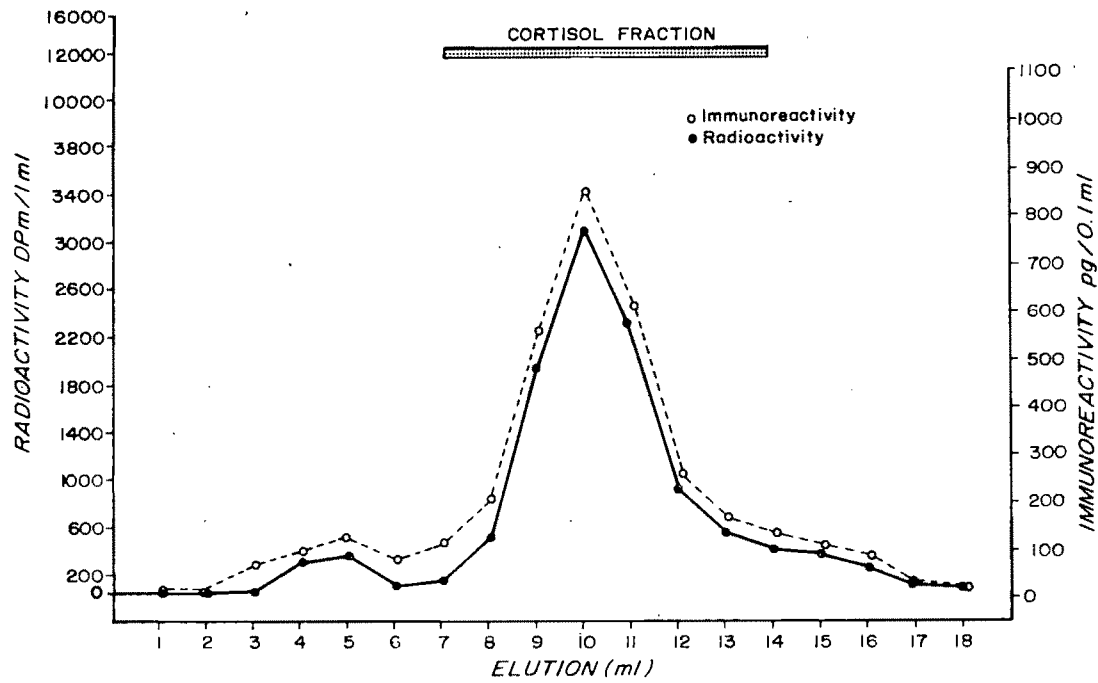


Fig. 1. Sephadex LH-20 co-chromatography elution patterns of plasma immunoreactive (o - - o) and radioactive (● — ●) F, F fractions: 7 to 14 ml.

Table I. Characteristics of steroid radioimmunoassays

|                                   | F                | DHEA             | A                | DHEA-S           |
|-----------------------------------|------------------|------------------|------------------|------------------|
| Slope of standard curve           | 0.86 ± 0.05      | 0.94 ± 0.04      | 0.95 ± 0.07      | 0.94 ± 0.04      |
| Range of linearity (pg)           | 100-1,000        | 20-1,000         | 20-1,000         | 20-1,000         |
| Slope of plasma samples           | 0.84 ± 0.05      | 0.93 ± 0.05      | 0.98 ± 0.07      | 0.93 ± 0.05      |
| Mass recovery:                    | y = 0.84x - 0.03 | y = 1.06x - 0.24 | y = 0.92x - 0.69 | y = 0.97x - 1.57 |
| Correlation coefficient           | 0.990            | 0.999            | 0.998            | 0.997            |
| Recovery of internal standard (%) | 82-92            | 75-85            | 85-95            | 70-80            |
| Sensitivity (ng/ml)               | 1.5              | 0.05             | 0.05             | 0.05             |
| Coefficients of variation (%):    |                  |                  |                  |                  |
| Intra-assay (%)                   | 5.4 (n = 5)      | 4.3 (n = 5)      | 5.1 (n = 5)      | 3.0 (n = 8)      |
| Inter-assay (%)                   | 10.4 (n = 18)    | 13.3 (n = 10)    | 12.1 (n = 10)    | 9.6 (n = 5)      |

Material and methods

**Surgical preparations.** Surgery was performed in eight pregnant ewes of mixed breed between 115 and 125 days of gestation. Gestational age was determined by x-ray evaluation of fetal bone maturation and breeding date if known. According to this method, the mean gestational length in eight animals averaged 147 days. Two polyvinyl catheters (0.54 mm outside diameter and 0.34 mm inside diameter) were implanted into the fetus as previously described<sup>9</sup>: one into a lateral tarsal vein for daily blood sampling and one into the subcutaneous tissue in the flank for the administration of BC (Sandoz). The BC was dissolved in 1,2-propanediol (1 mg/ml) and 1 mg was injected subcutaneously as a bolus every 12 hours. Following surgery, the animals were housed in cages, fed ad libitum, and inspected

daily. Four sheep served as controls for determination of the fetal PRL, F, DHEA, DHEA-S, and A concentrations, prior to and until the onset of spontaneous delivery. The remaining four fetuses, which made up the study group, were given BC, which was infused after a recovery period of 5 to 7 days until spontaneous delivery. In one of these animals (No. 060) 1,2-propanediol alone was infused for 4 days following the control period and before BC administration. Infusions of BC ranged between 21 and 11 days prior to delivery in various animals. Fetal blood pH measured at each sampling was above 7.33 in each animal throughout the experimental period. Fetal blood collected in heparin was separated immediately and the plasma was stored at -20° C until the time of hormonal assay.



**Radioiodination and radioimmunoassay.**

*PRL.* The radioiodination of ovine PRL (NIH P-S-12) was accomplished with the use of lactoperoxidase.<sup>10</sup> The radiolabeled hormone was chromatographically separated from free <sup>125</sup>I on a Bio-Gel P-10 column, then further purified with Sephadex G-75 column chromatography.

The PRL double-antibody radioimmunoassay is based on a modification of the method of Davis and associates<sup>11</sup> and has been reported previously from this laboratory.<sup>9</sup> All standards and unknown plasma determinations were performed in duplicate. The ovine prolactin standard curve was found to be linear between 5 and 100 ng. The slopes of the standard curve and various dilutions of fetal lamb plasma were parallel. The sensitivity of the assay, calculated as the 95% confidence level of the blank, averaged 1.5 ng/ml. The intra-assay and interassay coefficients of variation were 5.2% and 7.3%, respectively.

*Steroid hormones.* The procedures for each steroid assay are described in detail under each individual section. The specific characteristics of each assay are summarized in Table I.

*F assay.* Fetal sheep plasma F was determined according to a modification of the method of Abraham and associates.<sup>12</sup> Sheep plasma (0.2 ml) to which 1,000 cpm of <sup>3</sup>H-cortisol (New England Nuclear, Boston, Massachusetts) had been added to calculate procedural losses was extracted with 8 ml of ethyl acetate (Mallinckrodt, Inc., St. Louis, Missouri). The extract was evaporated to dryness and purified with Sephadex LH-20 column chromatography.

The column chromatography technique throughout this study was similar to that described by Auletta and associates.<sup>13</sup> In the F assay, the column was prepared by mixing 0.85 gm of Sephadex LH-20 (Pharmacia Fine Chemicals, Piscataway, New Jersey) with 4 ml of solvent (benzene:methanol, 80:20). The resultant slurry was poured into a minicolumn and washed with 5 ml of elution solvent (isooctane:benzene:methanol, 180:105:51). The dry sample was reconstituted with 0.2 ml of elution solvent and applied to the resin bed. The first 7 ml of eluate was discarded; the second 7 ml was collected in a scintillation vial for F determination. At the end of the radioimmunoassay, dextran-coated charcoal was used to separate free from antibody-bound F.

The F antiserum (RSL 145, Radioassay System Laboratories, Inc., Carson, California) was prepared against the conjugate cortisol-21-hemisuccinate-bovine serum albumin. The antisera, extensively characterized by the supplier, shows a significant cross-reaction (12%) with corticosterone only. However, after LH-20 col-

**Table II.** Gestational ages and duration of treatment in control and BC-infused sheep

| Group    | Sheep No. | Gestational age in days at delivery (mean ± SEM) | Duration of BC infusion (days) |
|----------|-----------|--------------------------------------------------|--------------------------------|
| Control  | 40        | 152                                              | —                              |
|          | 41        | 155                                              | —                              |
|          | 51        | 145                                              | —                              |
|          | 57        | 146                                              | —                              |
|          |           | 149.5 ± 2.4                                      |                                |
| Infusion | 60        | 146                                              | 13                             |
|          | 62        | 148                                              | 18                             |
|          | 64        | 152                                              | 21                             |
|          | 67        | 142                                              | 11                             |
|          |           | 147.0 ± 2.1                                      |                                |

umn chromatography, only 10% of the corticosterone present in the sample remains in the fraction used for F determination; therefore, the maximum interference in the assay would be 12% × 10% = 1.2%. Furthermore, since the F:corticosterone ratio in the fetal sheep has been noted to be 9.2 ± 0.61 (3), the interference of corticosterone in this F assay is negligible.

In order to test the specificity of this assay, 2.0 ml of fetal sheep plasma was cochromatographed with 20,000 cpm of <sup>3</sup>H-F. The effluent was collected in 1 ml fractions. Aliquots of fraction were assayed for radioactivity and for immunologic activity. The elution patterns for <sup>3</sup>H-F and endogenous unlabeled cortisol were similar (Fig. 1).

To test the accuracy of these assays, a mass recovery experiment was performed as follows: Increasing amounts of exogenous F, ranging from 1 to 25 ng, were added to 0.2 ml of fetal plasma. The amounts of F present in these samples were then measured by the present method. Each concentration was measured in quadruplicate. After correcting for endogenous steroid concentration, the data were analyzed by regression analysis. Table I contains the equation for this analysis and the other parameters for this assay.

*DHEA and A assays.* DHEA and A were extracted with 8 ml of petroleum ether (Mallinckrodt, Inc., St. Louis, Missouri) from 0.5 ml of fetal sheep plasma to which 1,000 cpm of <sup>3</sup>H-DHEA and <sup>3</sup>H-A (New England Nuclear) had been added to calculate recovery.

The extract was evaporated to dryness and purified by means of Sephadex LH-20 column chromatography. This column was prepared by mixing 1.5 gm of Sephadex with 6 ml of solvent (chloroform:hexane:ethanol, 50:50:1) and packing it into a minicolumn. The dried sample was applied to the column with 0.2 ml of solvent. The column was eluted with the same solvent. The first 1.5 ml of eluate was discarded; the second 2.5 ml fraction was collected for the determina-

Table III. The results of breakpoint calculations

|                                                          | <i>F</i>       |                     | <i>DHEA</i>    |                     | <i>DHEA-S</i>  |                     | <i>A</i>       |                     |
|----------------------------------------------------------|----------------|---------------------|----------------|---------------------|----------------|---------------------|----------------|---------------------|
|                                                          | <i>Control</i> | <i>Experimental</i> | <i>Control</i> | <i>Experimental</i> | <i>Control</i> | <i>Experimental</i> | <i>Control</i> | <i>Experimental</i> |
| Position of possible breakpoint (days prior to delivery) | 3              | 4                   | 18             | 19                  | 19             | 3                   | 3              | 3                   |
| Level of significance                                    | p < 0.001      | p < 0.001           | NS             | NS                  | NS             | NS                  | NS             | NS                  |

NS: Not significant.

tion of A; the third 0.5 ml was discarded, and the fourth 3.0 ml fraction was collected for the determination of DHEA. Both DHEA and A fractions were air dried.

The DHEA fraction was redissolved in 1.0 ml of ethanol; 0.2 ml and 0.4 ml aliquots were transferred to glass tubes (10 by 75 mm) and air dried. The remaining aliquot (0.4 ml) was counted for radioactivity to determine procedural losses. Phosphate buffer (0.1 ml) was added to the sample tubes to redissolve the DHEA. To these tubes 7,500 cpm of  $^3\text{H}$ -DHEA in 0.1 ml of buffer and 0.1 ml of diluted (1:100) antiserum were added. The tubes were agitated and incubated overnight at 4° C. Following incubation, 1.0 ml of dextran-coated charcoal was added to separate free from antibody-bound  $^3\text{H}$ -DHEA. The radioimmunoassay for A was similar to that for DHEA.

The DHEA and A antisera were also purchased from Radioassay System Laboratories. DHEA and A antisera (RSL 149, RSL 144) were prepared against the DHEA-3 $\beta$ -monohemisuccinate-bovine serum albumin and androstenedione-carboxymethylether-bovine serum albumin, respectively. The DHEA antiserum only cross reacted significantly with A (12.5%), while the A antisera cross reacted significantly only with DHEA (4.2%); therefore, it was necessary to separate one from the other by means of Sephadex column chromatography prior to the radioimmunoassay.

In order to confirm the specificity of this Sephadex column chromatography technique, 3.0 ml of sheep fetal plasma was cochromatographed with 30,000 cpm of  $^3\text{H}$ -DHEA and  $^3\text{H}$ -A. The effluent was collected in 0.5 ml of fractions; 0.2 ml of each fraction was counted for radioactivity, and the rest of the aliquots were used for DHEA and A immunologic activity assays. The elution patterns for  $^3\text{H}$ -A and unlabeled A were similar. DHEA also showed similar curves for radioactivity and immunoactivity. Specific characteristics of these assays are shown in Table I.

**DHEA-S assay.** DHEA-S was subjected to solvolysis prior to chromatography according to the method of Nieschlag and associates.<sup>14</sup> Plasma samples (0.2 ml) were mixed with 1.8 ml of a saturated sodium chloride

solution and 1,000 cpm of  $^3\text{H}$ -DHEA-S in a centrifuge tube. The sulfate was extracted with 5 ml of ethyl acetate and transferred to glass-stoppered centrifuge tubes to which 0.5 ml of glacial acetic acid was added. All tubes were kept overnight in a water bath at 75° C. After solvolysis, the reaction mixture was dried and the residue was applied to a Sephadex column and assayed as described in the free DHEA assay.

Since ethyl acetate will extract both DHEA-S and free DHEA originally present in the plasma sample, the results obtained measure total DHEA (free plus sulfoconjugated). Since the recovery of free  $^3\text{H}$ -DHEA and  $^3\text{H}$ -DHEA-S were found to be identical throughout the extraction and solvolysis processes, the concentration of DHEA-S present in the samples was determined as follows:

$$\text{DHEA-S} = \text{total DHEA-DHEA (determined from previous DHEA assay)} \times \frac{\text{molecular weight of DHEA-S}}{\text{molecular weight of DHEA}}$$

Specific characteristics of this assay are shown in Table I.

**Statistical analysis.** Results for each hormone concentration obtained in each sheep fetus were plotted according to the number of days prior to delivery. Mean concentrations were then calculated for both the control and BC-treated groups. These data were examined for the presence of a "breakpoint" which might indicate a shift in the trend. Visual analysis indicated that most hormonal concentrations showed a possible breakpoint at the last 3 days prior to delivery.

An algorithm was used to determine the position of the point where the slope of the line changed (breakpoint). It was as follows: All data points were divided into two segments and straight lines were fitted separately to each. Residual sums of squares were computed independently for each segment and added to give a total residual sum of squares. This was done from the third to the n-2 point inclusive. That point giving a minimum total sum of squares was a possible breakpoint. The two lines thus obtained were tested to determine if they were statistically significantly different by means of a standard F test.<sup>15</sup>

In a few cases, visual analysis of the data indicated



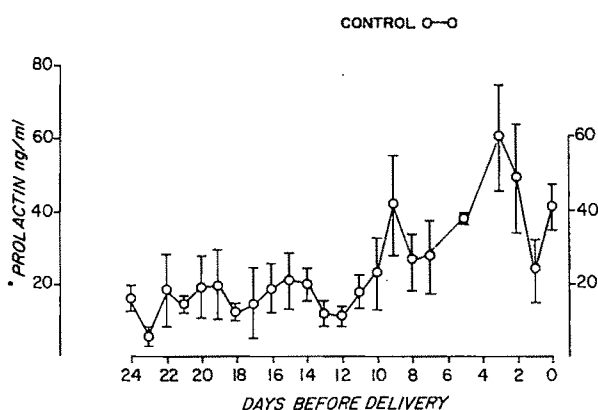


Fig. 2. Ovine fetal plasma PRL (mean  $\pm$  SEM) concentrations in control group ( $n = 4$ ) prior to delivery (day 0).

that their might be a breakpoint within 2 or 3 days at the beginning of the series long before delivery. It appeared that the later period after the breakpoint and closer to the time of delivery demonstrated a flat line (zero slope). In such cases, all of the values for these hormones were tested for "outliers" according to the criterion  $z = (X - \bar{X}) \div SD$ .<sup>16</sup> This method was used to identify individual values that differed significantly from the line of best fit.

To determine the effect of BC treatment on steroidogenesis, a pair of control and experimental curves for each steroid was compared. A dummy variable technique<sup>15</sup> was then applied to test for the difference in the coefficients (a quadratic model) between control and experimental data. If no significant difference was found, the shapes of the curves were the same, indicating that the BC treatment had no effect on steroidogenesis.

## Results

In the one animal who received 1,2-propanediol for 4 days prior to BC infusion, there was no difference between hormonal concentrations during the control period and during administration of the vehicle solution.

**Duration of gestation and fetal outcome.** All ewes delivered live, healthy lambs spontaneously at term. The mean lengths of gestation of the control ewes and ewes whose fetuses received BC were similar ( $149.5 \pm 2.4$  and  $147.0 \pm 2.1$  days, respectively) (Table II).

**Results of breakpoint calculation.** The results of the breakpoint calculation are shown in Table III. F showed significant breakpoints at 3 and 4 days prior to delivery for control and experimental groups, respectively. All other breakpoints were found not to be statistically significant.

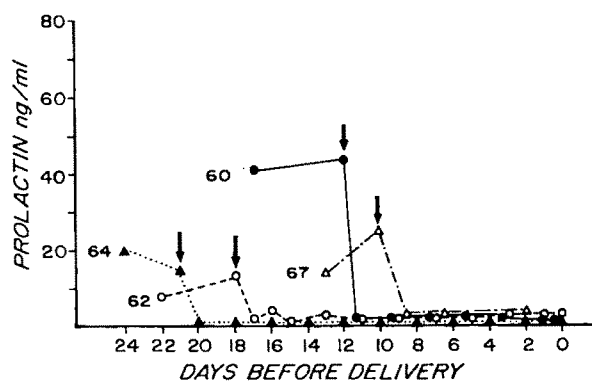


Fig. 3. Ovine fetal plasma PRL concentration in the BC-treated (1 mg/12 hours) group ( $n = 4$ ).  $\downarrow$  = Beginning of treatment.

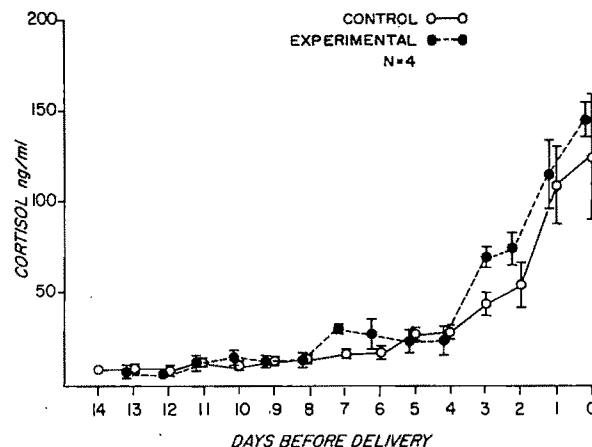


Fig. 4. Ovine fetal plasma F concentration (mean  $\pm$  SEM) in control (o—o) and BC-treated (•—•) groups.

**PRL.** The plasma concentration of PRL increased in control fetuses from  $16.2 \pm 3.3$  ng/ml at 24 days before delivery to  $41.0 \pm 6.3$  ng/ml at birth (Fig. 2). In the BC-infused group, an immediate decrease in PRL concentration was noted within 24 hours of administration of BC and values remained below 3 ng/ml until delivery (Fig. 3).

**F.** According to the breakpoint calculation, F showed a sharp increase at 3 and 4 days prior to delivery for control and experimental groups, respectively (Table III and Fig. 4). In the control group, F increased from  $6.6 \pm 0.2$  ng/ml at 12 days before delivery to  $124.7 \pm 35.4$  ng/ml on the day of parturition. In the BC-infusion group, the values increased from  $8.7 \pm 1.6$  to  $145.4$  ng/ml during the same period. A very important finding is that after BC administration no significant change in the F concentration was observed. The dummy variable techniques were applied to the control and experimental data. Both curves could be best fitted

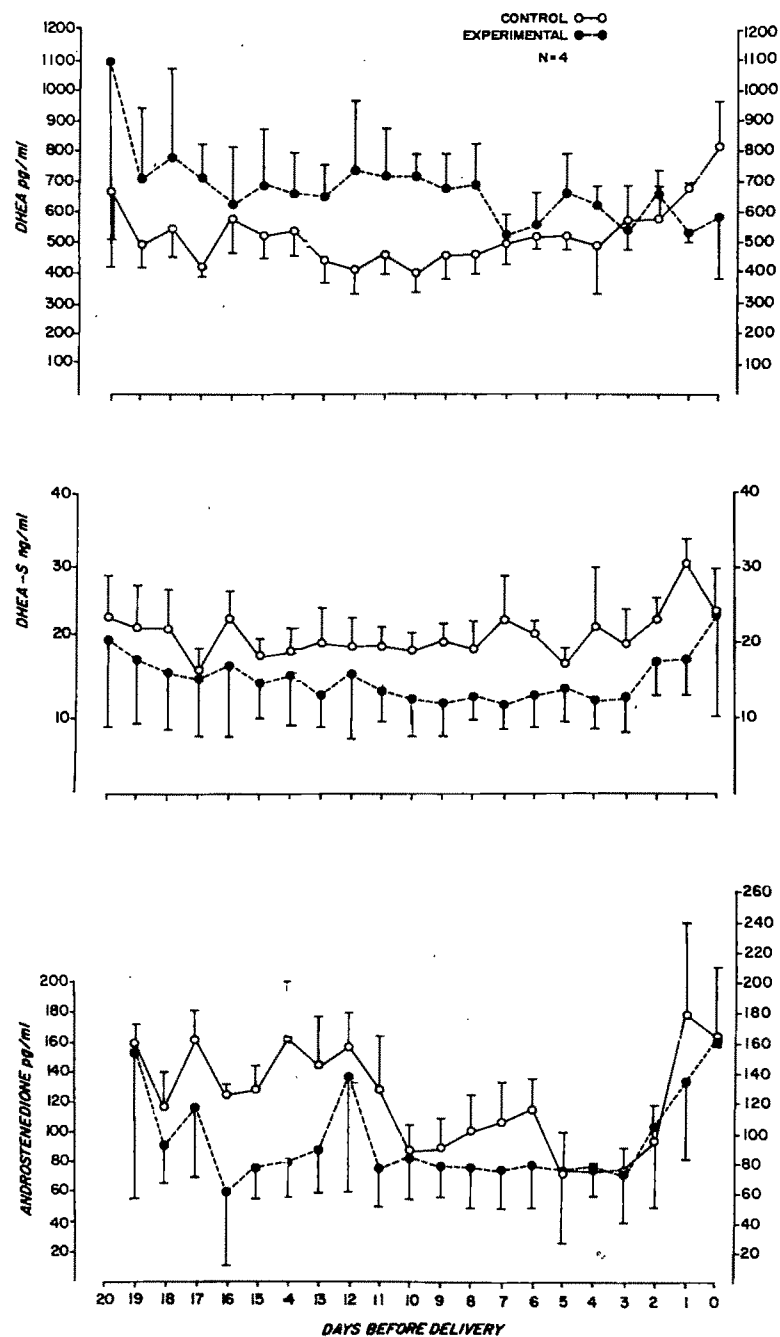


Fig. 5. Ovine fetal plasma DHEA, DHEA-S, and A concentrations (mean  $\pm$  SEM) in control (o—o) and BC-treated (●---●) groups.

with parabolic equations. At 99.9% of the significance level, both curves were similar. These results implied that the BC treatment did not modify the secretion of F.

**DHEA.** The concentrations of DHEA in both control and experimental groups were relatively unchanged throughout the last 20 days of pregnancy (Fig. 5). All data points were fluctuating within a range of 400 to 800 pg/ml. The "outlier" test showed that the control

group had a tendency to increase in the last 48 hours before delivery ( $p < 0.01$ ). However, the BC-treated group had stable concentrations throughout pregnancy. No statistical difference between control and experimental groups was found.

**DHEA-S.** The concentration of DHEA-S in the control group was quite stable (14 to 25 ng/ml) until the last 24 hours prior to parturition. Based on the "out-



lier" test it increased slightly ( $p < 0.01$ ), as shown in Fig. 5. In the BC-infused group, the mean concentration of DHEA-S increased from 12 to 24 ng/ml at 3 days prior to parturition. The dummy variable technique also showed no significant difference between control and experimental groups throughout the last 20 days of gestation.

A. The concentration of A increased at 3 days prior to parturition in both the control and experimental groups (Fig. 5). In the last 3 days, A in the control group increased from 76 to 162 pg/ml while in the experimental group it increased from 73 to 166 pg/ml. No "outlier" could be identified for either group. When the control and BC-treated groups were tested by the dummy variable technique, no statistical difference was noted.

#### Comment

Since many steroid radioimmunoassays originally characterized for human plasma were not applicable for ovine studies, we developed and validated the assay techniques to measure fetal sheep plasma F, DHEA, DHEA-S, and A in this study. Our data suggest that the fetal PRL levels increase 5 days before parturition (28 to 43 ng/ml, Fig. 2). During the same period, F concentrations rise from 22 to 125 ng/ml. These results are similar to the data reported by Abel and associates.<sup>8</sup> Fetal DHEA-S concentrations ranging between 12 and 24 ng/ml (Fig. 5) are compatible with the data of Ballard and associates.<sup>17</sup> Individual sheep and day-to-day variations were also observed in their investigation. Our data (Fig. 5) show that DHEA and A concentra-

tions were 500 to 800 and 80 to 160 pg/ml, respectively. Colas and Curet<sup>18</sup> found that the DHEA and A levels were relatively stable during the last 9 days of gestation and that DHEA levels were higher than the A level. However, their DHEA and A values were higher than those obtained in our experiments.

Results from this study indicate that administration of BC to the fetus significantly decreases fetal PRL concentrations. However, in spite of this, there was no difference in the length of gestation between the normal and BC-infused groups. This finding is in agreement with the work published by Lowe and associates.<sup>19</sup> Since no correlation was found between PRL concentrations and the length of gestation, it is difficult to postulate a role for fetal PRL in the initiation of labor. Also the rise in fetal F, essential for the initiation of labor, was similar in timing and degree in both groups. This finding is consistent with a report by Lowe and associates,<sup>20</sup> who failed to observe any effect of PRL infusion on F in fetal sheep. Furthermore, Ballard and associates<sup>17</sup> recently reported that infusion of ovine PRL had no effect on fetal plasma DHEA-S concentrations. All of these studies indicate that ovine fetal PRL is not required for F production or thus for initiation of labor. In conclusion, our results indicate that fetal PRL does not affect the initiation of labor or the concentration of plasma F, DHEA, DHEA-S, and A in fetal sheep.

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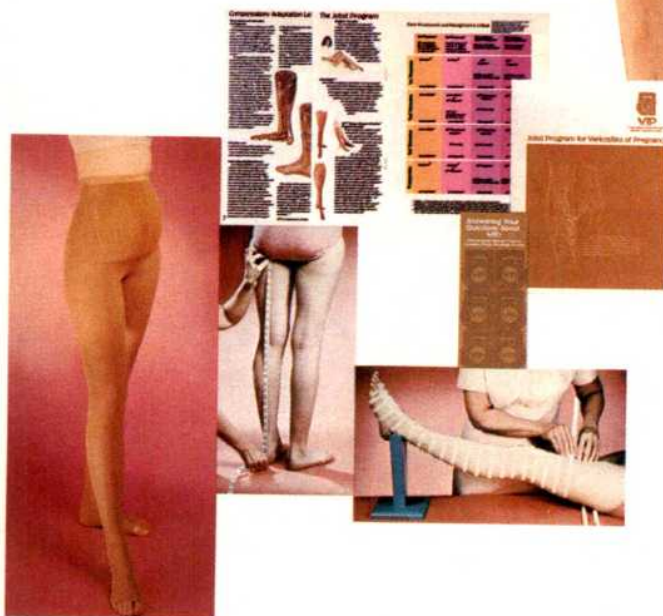
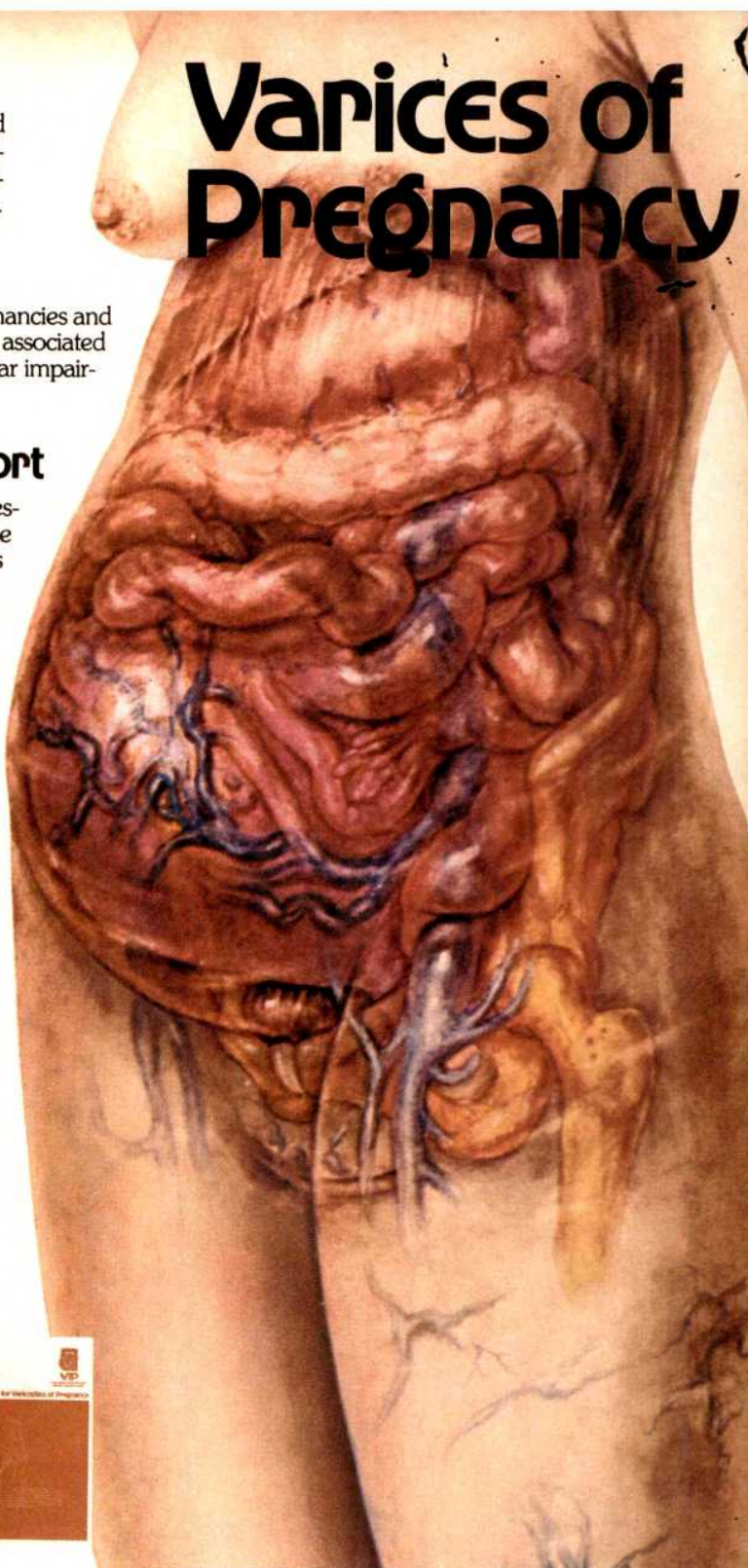
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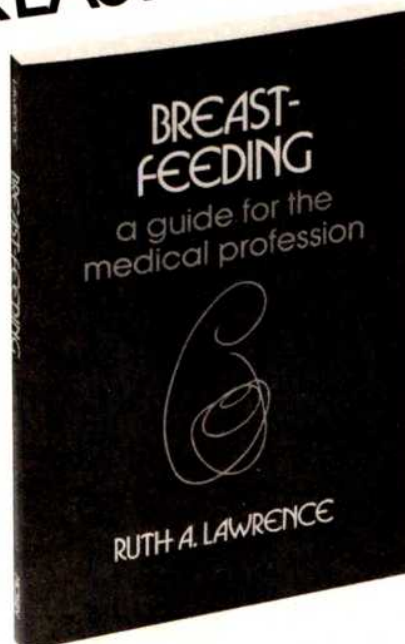
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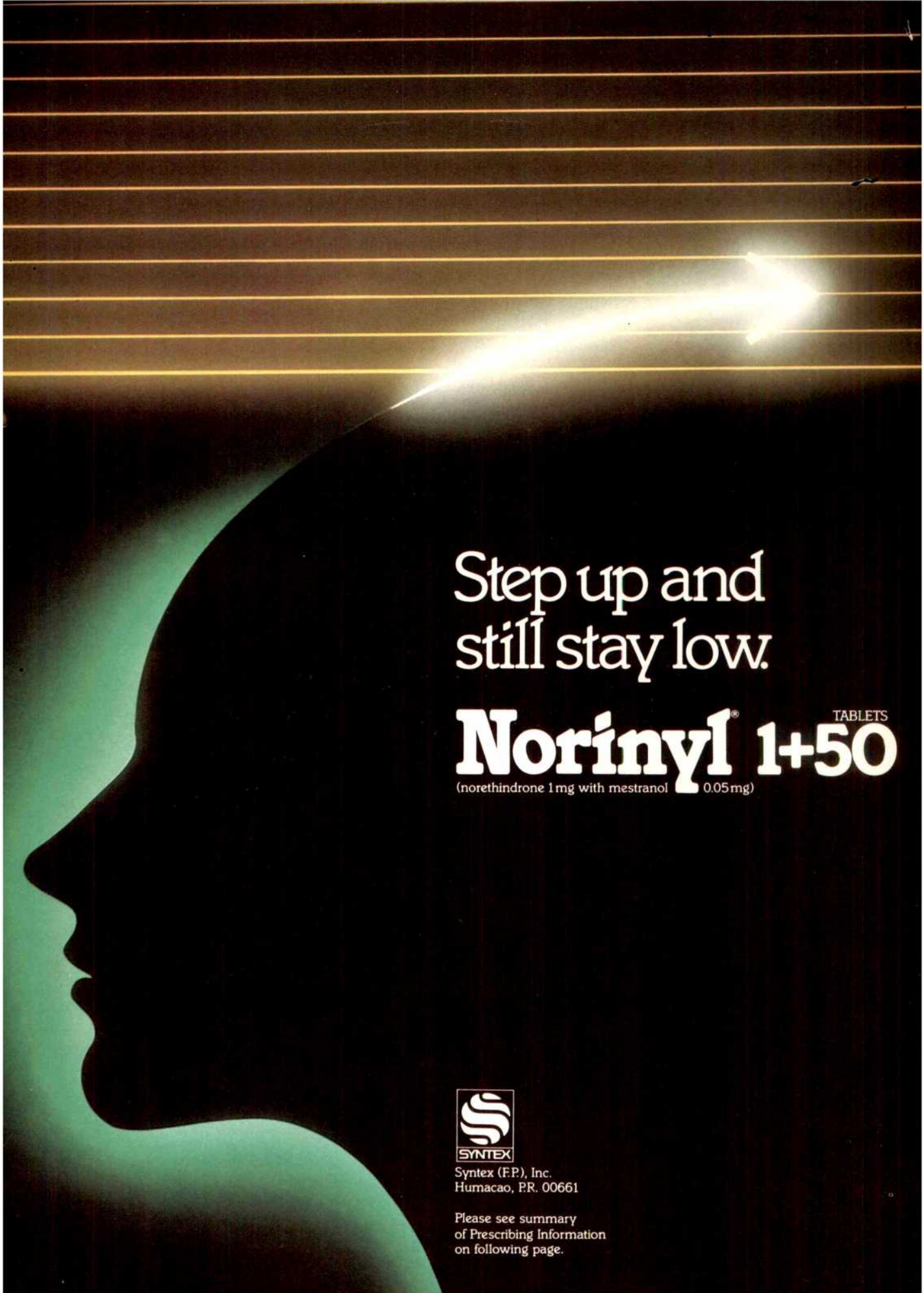


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1. **Thromboembolic Disorders and Other Vascular Problems:** An increased risk of thromboembolic and thrombotic disease associated with the use of O.C.s is well established. One British study demonstrated an increased relative risk for fatal venous thromboembolism; two British and three American studies demonstrated an increased relative risk for non-fatal venous thromboembolism. These studies estimate that users of O.C.s are 4 to 11 times more likely than nonusers to develop these diseases without evident cause.

**TABLE 1.** The annual number of deaths associated with control of fertility and no control per 100,000 nonsterile women by regimen of control and age of women are\*

The risk of thromboembolic and thrombotic disease associated with O.C.s increases with age after approximately age 30 and, for myocardial infarction, is further increased by hypertension, hyperlipidemia, obesity, diabetes, or history of preeclamptic toxemia and especially by cigarette smoking. Based on

|                                               | Age | Below 30 | 30-39 | 40+ |
|-----------------------------------------------|-----|----------|-------|-----|
| Heavy smokers                                 | C   | B        | A     |     |
| Light smokers                                 | D   | C        | B     |     |
| Nonsmokers<br>(No predisposing conditions)    | D   | C, D     | C     |     |
| Nonsmokers<br>(other predisposing conditions) | C   | C, B     | B, A  |     |

A—Use associated with very high risk. C—Use associated with moderate risk.  
D—Use associated with high risk. D—Use associated with low risk.

bladder disease in users of O.C.s or estrogens. In one study, an increased risk appeared after 2 years of use and doubled between 4 and 6 years of use. In another study, the risk was about equal after 6 and 12 months of use. 7. **Carbohydrate and Lipid Metabolism:** Effects on blood glucose tolerance have been observed in a significant percentage of patients on O.C.s. For this reason, prediabetic and diabetic patients should be carefully observed while receiving O.C.s. An increase in triglycerides and total phospholipids has been observed in patients receiving O.C.s. The clinical significance of this finding is unknown. 8. **Elevated Blood Pressure:** An increase in blood pressure has been reported with O.C. use. In some women, hypertension may occur within a few months of beginning O.C. use. In the first year of use, the prevalence of women with hypertension is low in users and may be higher in nonusers. After a second year of use, the prevalence in users increases, however, with longer duration of use, and the ratio of users to nonusers becomes two and a half to three times the reported prevalence in the fifth year of use. Age is also strongly correlated with the development of hypertension in O.C. users. Women with a history of elevated blood pressure (hypertension), preexisting renal disease, a history of toxemia or elevated blood pressure during pregnancy, a familial tendency to hypertension or its consequences, or a history of excessive weight gain or fluid retention during the menstrual cycle may be more likely to develop elevation of blood pressure when given O.C.s and, therefore, should be monitored closely. Even though elevated blood pressure may be reversible after discontinuation of O.C.s, continued surveillance should not be ignored and close surveillance is indicated, particularly in women with other risk factors for cardiovascular disease or stroke. High blood pressure may or may not persist after discontinuation of the O.C. **Headache:** The onset or exacerbation of migraine or development of headache of a new pattern which is recurrent, persistent, or severe, requires discontinuation of O.C.s and evaluation of the cause. 10. **Bleeding Irregularities:** Breakthrough bleeding, spotting, and missed menses are frequent reasons for patients discontinuing O.C.s. In breakthrough bleeding, as in all cases of irregular uterine bleeding patterns, nonfunctional causes should be borne in mind. Undiagnosed persistent or recurring abnormal vaginal bleeding, especially if associated with amenorrhea at the time of treatment, may indicate pregnancy; adequate diagnostic measures are indicated to rule out pregnancy or malignancy. If pathology has been excluded, time or a change to another formulation may solve the problem. Changing to an O.C. with a higher estrogen content, while potentially useful in minimizing menstrual irregularity, should be done only if necessary since this may increase the risk of thromboembolic disease. Women with a past history of oligomenorrhea or secondary amenorrhea or young women without regular cycles may have a tendency to remain anovulatory or to become amenorrheic after discontinuation of O.C.s. The break-through bleeding pattern may continue because of this possibility and encouraged to use other contraceptive methods. Possibility of prolonged anovulation, possibly prolonged, may also occur in women without previous irregularities. A higher incidence of galactorrhea and of pituitary tumors (e.g., adenomas) has been associated with amenorrhea in former users compared with nonusers. One study reported a 16-fold increased prevalence of pituitary prolactin-secreting tumors among patients with postpill amenorrhea when galactorrhea was present. 11. **Fertility:** There is evidence of impairment of fertility in women discontinuing O.C.s in comparison with those discontinuing other methods of contraception. The impairment appears to be temporary. As long as the breaking pattern continues, there is no loss of this possibility and encouraged to use other contraceptive methods. Possibility of prolonged anovulation, possibly prolonged, may also occur in women without previous irregularities. A higher incidence of galactorrhea and of pituitary tumors (e.g., adenomas) has been associated with amenorrhea in former users compared with nonusers. One study reported a 16-fold increased prevalence of pituitary prolactin-secreting tumors among patients with postpill amenorrhea when galactorrhea was present. 12. **Ectopic Pregnancy:** Ectopic as well as intrauterine pregnancy may occur in contraceptive failures. However, in progesterone-only O.C. failures, the ratio of ectopic to intrauterine pregnancies is higher than in women who are not receiving O.C.s, since the drugs are more effective in preventing intrauterine than ectopic pregnancies. Therefore, if O.C.s fail in the postpartum period many months later with lactation, there is a decreased risk of ectopic pregnancy. The quality of the breast milk. Furthermore, a small fraction of the hormonal agents in O.C.s has been identified in the milk of mothers receiving these drugs. The effects, if any, on the breast fed child have not been determined. If feasible, the use of O.C.s should be deferred until the infant has been weaned.

**PRECAUTIONS GENERAL:** A complete medical and family history should be taken prior to the initiation of O.C.s. The pretreatment and periodic physical examinations should include special reference to blood pressure, breasts, abdomen and pelvic organs, including Papanicolaou smear and recommended ancillary tests. As with any rule, O.C.s should not be prescribed for longer than 1 year without another physical examination being performed. 2. Under the influence of estrogen-progesterone preparations, preexisting uterine leiomyomata may increase in size. 3. Patients with a history of psychic depression should be carefully observed and the drug discontinued if depression recurs to a serious degree. Patients becoming significantly depressed while taking O.C.s should stop the medication and use an alternate method of contraception in an attempt to determine whether the symptom is drug related. 4. O.C.s may cause some degree of fluid retention. They should be discontinued with caution, and only with careful monitoring, in patients with conditions which might be aggravated by fluid retention, such as convulsive disorders, migraine symptoms, asthma, or cardiac, hepatic or renal insufficiency. 5. Patients with a past history of jaundice during pregnancy have an increased risk of recurrence of jaundice while receiving O.C. therapy. If jaundice develops in any patient receiving such drugs, the medication should be discontinued. 6. Steroid hormones may be poorly metabolized in patients with impaired liver function and should be administered with caution in such patients. 7. O.C. users may have disturbances in normal tryptophan metabolism which may result in a relative pyridoxase deficiency. The clinical picture resembles that caused by true vitamin B<sub>6</sub> deficiency. It may be prevented by O.C. therapy. Since the pregnant woman predisposed to the development of folate deficiency and the incidence of folate deficiency increases with increasing gestation, it is possible that if a woman becomes pregnant shortly after stopping O.C.s, she may have a greater chance of developing folate deficiency and complications attributed to this deficiency. 9. The pathologist should be advised of O.C. therapy when relevant specimens are submitted. 10. Certain endocrine and liver function tests and blood components may be affected by estrogen-containing O.C.s. For example, a Increased sulfonylurea hypoglycemic response, increased prothrombin and factors VII, VIII, IX, X, XI, XII, XIII, fibrinogen, and plasminogen activator (tissue plasminogen activator). C. Increased thyroid binding globulin (TBG) leading to increased circulating total thyroid hormone, as measured by protein-bound iodine (PBI), T<sub>4</sub> by column, or T<sub>4</sub> by radioimmunoassay. Free T<sub>3</sub> resin uptake is decreased, reflecting the elevated TBG. Free T<sub>4</sub> concentration is unaltered. D. Decreased pregnandiol excretion. E. Reduced response to metoprolol test. **DRUG INTERACTIONS:** O.C.s may be rendered less effective by virtue of drug interaction with rifampin, isoniazid, ampicillin, tetracycline, neomycin, penicillin V, oral contraceptives, phenytoin, barbiturates, p-aminosalicylic acid, salicylates, analgesics, tranquilizers, and antimetabolic paracetamol. C. May alter the effectiveness of other types of drugs, such as oral anticoagulants, anticonvulsants, tricyclic antidepressants, antihypertensive agents (e.g., guanethidine), vitamins and hypoglycemic agents. **CARCINOGENESIS AND WARNINGS:** **PREGNANCY:** Pregnancy category X. See Contraindications and Warnings. **NURSING MOTHERS:** See Warnings. **ADVERSE REACTIONS:** An increased risk of the following serious adverse reactions has been associated with the use of O.C.s (see Warnings): thrombophlebitis, thrombosis, pulmonary embolism, coronary thrombosis, cerebral thrombosis, mesenteric thrombosis, thrombotic thrombocytopenic purpura, gestational thrombosis, disease, congenital anomalies, neuro-ocular lesions, e.g., retinal thrombosis and optic neuritis. The following adverse reactions have been reported in patients receiving O.C.s and are believed to be drug related: bleeding irregularities (breakthrough bleeding, spotting, missed menses during treatment, amenorrhea after treatment), gastrointestinal symptoms (nausea, vomiting, bloating, abdominal cramps), dysmenorrhea, infertility after discontinuance of treatment, edema, chloasma or melasma which may persist after drug is discontinued, contact lens changes in corneal curvature (steepening) change in weight (increase or decrease), change in cervical erosion and cervical secretion, possible diminution in lactation when given immediately postpartum, cholestatic jaundice, migraine, increase in size of uterine leiomyoma, rash (allergic), mental depression, reduced tolerance to carbohydrates, vaginal candidiasis, prolactin-secreting pituitary tumors. The following adverse reactions have been reported in users of O.C.s, and the association has been neither confirmed nor refuted: pseudotumor-like syndrome, cataracts, changes in color vision, changes in appetite, changes in taste, changes in hair growth, nervousness, dizziness, hirsutism, loss of hair, erythema multiforme, erythema nodosum, hemorrhagic eruption, vaginitis, porphyria, impaired renal function.

**INFORMATION FOR THE PATIENT (See Patient Package Insert)**

**INFORMATION FOR THE PATIENT** (See Patient Package Insert)



## Articles to appear in early issues

### **Postcoital testing in relation to circulating sperm-agglutinating antibodies in women**

Jan Friberg, M.D., Ph.D.

*Brooklyn, New York*

### **The carbon dioxide laser in cervical intraepithelial neoplasia: A five-year experience in treating 230 patients**

Byron J. Masterson, M.D., Kermit E. Krantz, M.D., John W. Calkins, M.D., Javier F. Magrina, M.D., and Robert P. Carter, M.D.

*Kansas City, Kansas*

### **Changes in the milk composition of nonpuerperal women**

Jerzy K. Kulski, Ph.D., Peter E. Hartmann, Ph.D., Warren J. Saint, B.Med.Sci.,

Patrick F. Giles, M.Sc., M.B., F.R.C.O., and Donald H. Gutteridge, M.B., F.R.A.C.P.

*Nedlands, Western Australia*

### **The ultrasonographic evaluation of large cystic pelvic masses**

Eric R. Rosenberg, M.D., and William S. Trought, M.D.

*Durham, North Carolina*

### **Sonographic abdominal circumference: Dynamic versus static imaging**

Carl P. Weiner, M.D., Rudy E. Sabbagha, M.D., Ralph K. Tamura, M.D., and

Sharon DalCompo, R.T., R.D.M.S.

*Columbus, Ohio, and Chicago, Illinois*

### **False reactive nonstress tests in postterm pregnancies**

Fred S. Miyazaki, M.D., F.A.C.O.G., and Brian A. Miyazaki

*Los Angeles and Irvine, California*

### **Increased gentamicin dosage requirements: Rapid elimination in 249 gynecology patients**

Darwin E. Zaske, Pharm.D., F.C.P., Robert J. Cipolle, Pharm.D., Richard G. Strate, M.D., F.A.C.S., and William F. Dickes, M.D.

*St. Paul, Minnesota*

### **Low-density lipoprotein as a potential vehicle for chemotherapeutic agents and radionucleotides in the management of gynecologic neoplasms**

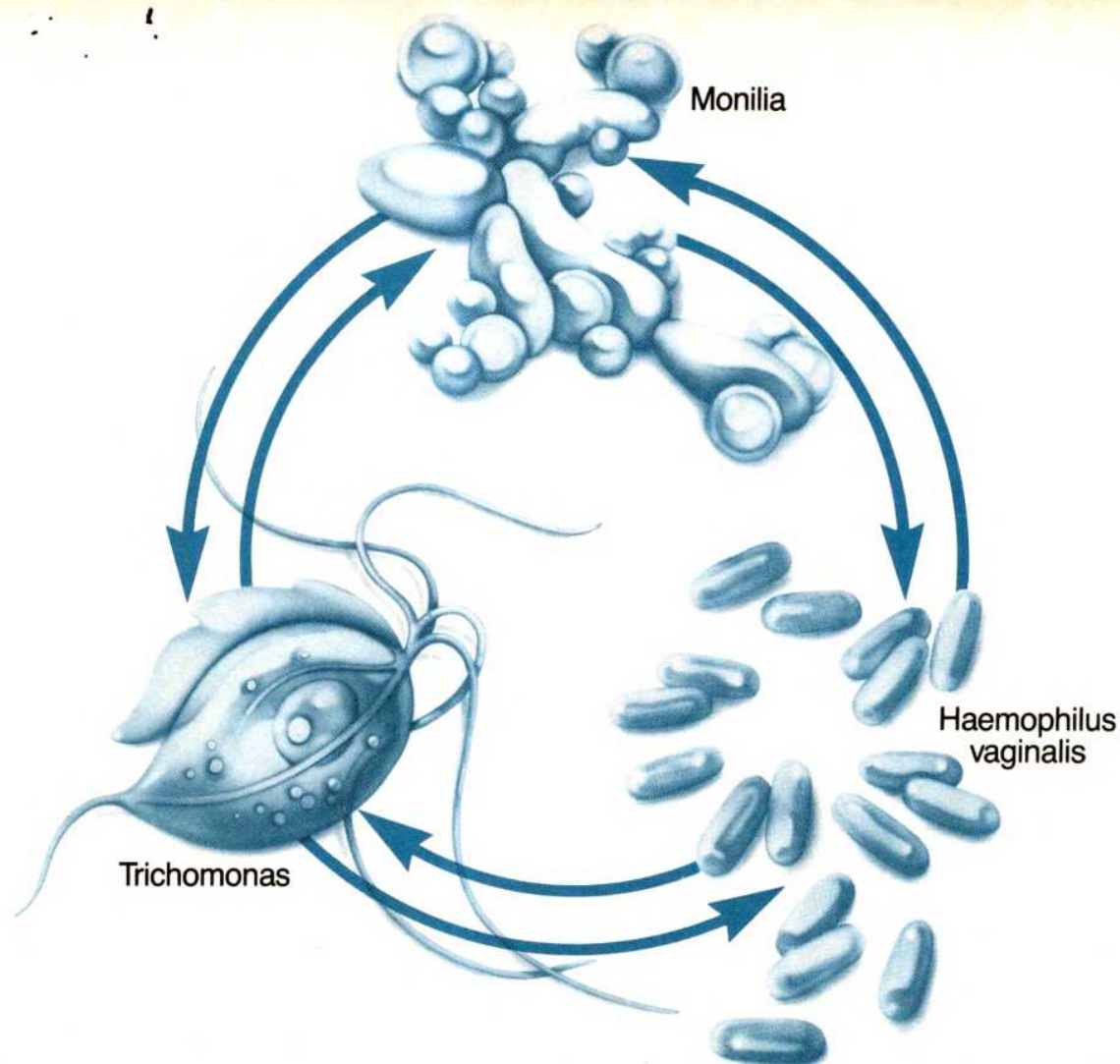
David Gal, Masao Ohashi, Paul C. MacDonald, Herbert J. Buchsbaum, and Evan R. Simpson

*Dallas, Texas*

### **Daily and circadian variations in serum free testosterone levels are not clinically significant**

Ronald C. Strickler, M.D., Walter G. Wiest, Ph.D., Rudi Borth, D.Sc., and C. Allan Woolever, M.D.

*St. Louis, Missouri, and Toronto and Hamilton, Ontario, Canada*



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**AT HOME**

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- In the office, swab the cervix and vulvovaginal area with BETADINE Solution.
- Prescribe BETADINE Vaginal Gel (povidone-iodine), one applicatorful of which is to be inserted each night.
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- After two weeks
  - Patient returns for an office visit.
  - In more resistant cases, the regimen may be continued through 1 or 2 cycles, if necessary.

**BETADINE® Vaginal Gel**  
(povidone-iodine)

**Brief Summary**

For use in the treatment of *Trichomonas vaginalis* vaginitis, monilial vaginitis and nonspecific vaginitis.

Based on a review of this drug by the National Academy of Sciences—National Research Council and/or other information, FDA has classified the indications as follows: possibly effective for use in the management of *trichomonas vaginalis* vaginitis, monilial vaginitis and non-specific vaginitis. Final classification of the less-than-effective indications requires further investigation.

**Precaution**

If irritation, redness or swelling develops, discontinue use. The rare individual with history of iodine sensitization should not use this product, pending further investigation.

**Purdue Frederick**

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## PERINATOLOGIST

St. Joseph's Hospital and Medical Center in Phoenix, Arizona, a 591-bed teaching hospital with approved residency in obstetrics and gynecology, and a designated regional perinatal center with an active high risk maternal transport system, is seeking a full-time perinatologist for its Division of Reproductive Medicine. Applicant must be Board certified in obstetrics and gynecology and eligible or certified in maternal and fetal medicine.

All correspondence confidential. Send inquiries and curriculum vitae to:

William D. Nicely  
Vice President—Patient Services  
St. Joseph's Hospital  
and Medical Center  
P.O. Box 2071  
Phoenix, Arizona 85001

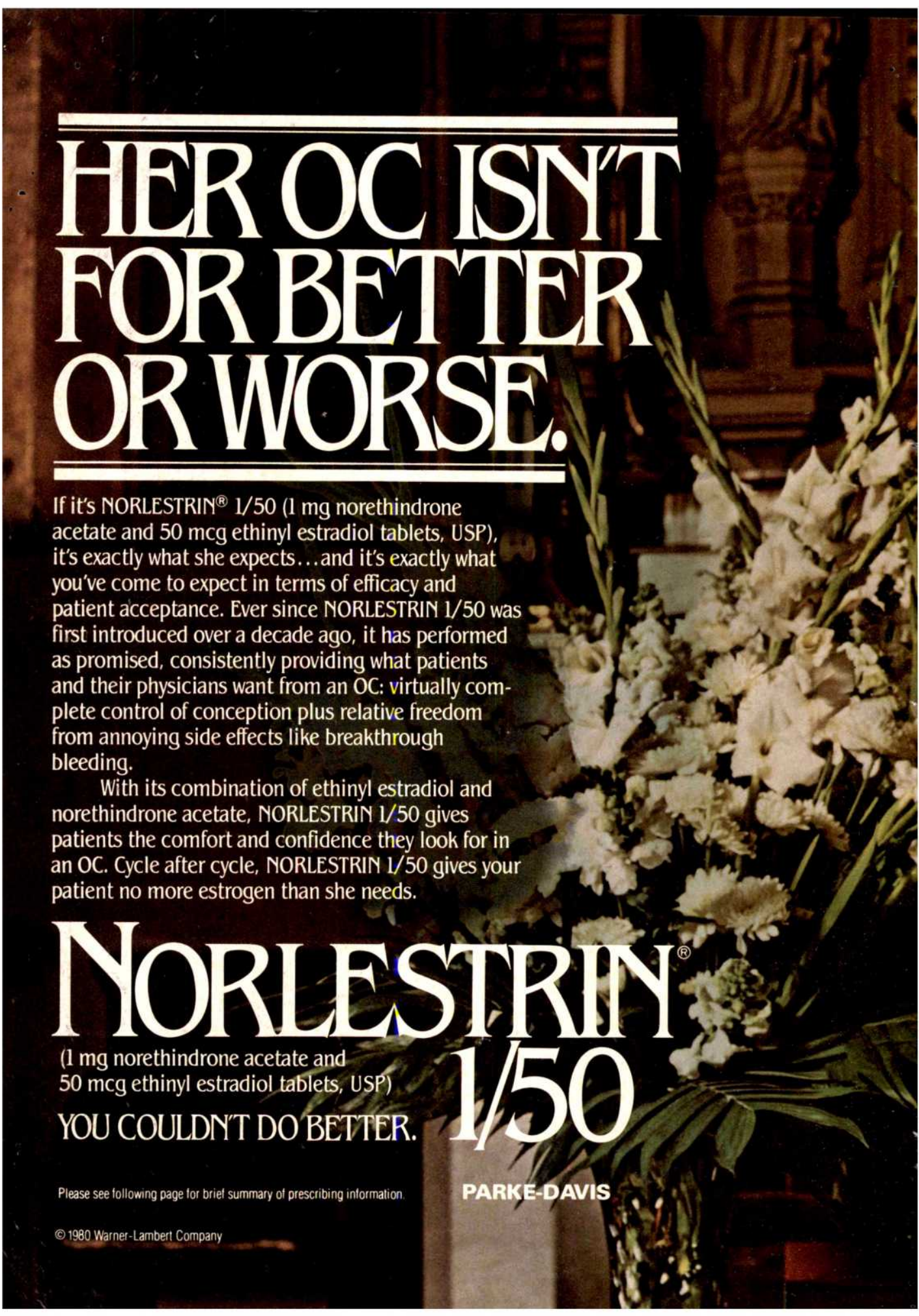
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# HER OC ISN'T FOR BETTER OR WORSE.

If it's NORLESTRIN® 1/50 (1 mg norethindrone acetate and 50 mcg ethinyl estradiol tablets, USP), it's exactly what she expects...and it's exactly what you've come to expect in terms of efficacy and patient acceptance. Ever since NORLESTRIN 1/50 was first introduced over a decade ago, it has performed as promised, consistently providing what patients and their physicians want from an OC: virtually complete control of conception plus relative freedom from annoying side effects like breakthrough bleeding.

With its combination of ethinyl estradiol and norethindrone acetate, NORLESTRIN 1/50 gives patients the comfort and confidence they look for in an OC. Cycle after cycle, NORLESTRIN 1/50 gives your patient no more estrogen than she needs.

# NORLESTRIN®

(1 mg norethindrone acetate and  
50 mcg ethinyl estradiol tablets, USP)

# 1/50

YOU COULDN'T DO BETTER.

Please see following page for brief summary of prescribing information.

PARKE-DAVIS

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## NORLESTRIN® 1/50

(norethindrone acetate and ethinyl estradiol tablets, USP) See section under Special Notes on Administration and HOW SUPPLIED.

**Before prescribing, please see full prescribing information. A Brief Summary follows:** Each yellow tablet contains: norethindrone acetate (17 alpha-ethinyl-19-nortestosterone acetate), 1 mg; ethinyl estradiol (17 alpha-ethinyl-1,3,5(10)-estratriene-3, 17 beta-diol), 50 mcg.

### DESCRIPTION

Norlestrin 1/50 Products are progestogen-estrogen combinations.

### INDICATIONS AND USAGE

Norlestrin 1/50 Products are indicated for the prevention of pregnancy in women who elect to use oral contraceptives as a method of contraception.

In clinical trials with Norlestrin 1/50 involving 25,983 therapy cycles, there was a pregnancy rate of 0.05 per 100 woman years.

**Dose-related risk of thromboembolism from oral contraceptives:** Studies have shown a positive association between the dose of estrogens in oral contraceptives and the risk of thromboembolism. It is prudent and in keeping with good principles of therapeutics to minimize exposure to estrogen. The oral contraceptive prescribed for any given patient should be that product which contains the least amount of estrogen that is compatible with an acceptable pregnancy rate and patient acceptance.

### CONTRAINDICATIONS

1. Thrombophlebitis or thromboembolic disorders
2. A past history of deep-vein thrombophlebitis or thromboembolic disorders
3. Cerebral vascular or coronary artery disease
4. Known or suspected carcinoma of the breast
5. Known or suspected estrogen-dependent neoplasia
6. Undiagnosed abnormal genital bleeding
7. Known or suspected pregnancy (See WARNING No. 5.)
8. Benign or malignant liver tumor which developed during the use of oral contraceptives or other estrogen-containing products

### WARNINGS

**Cigarette smoking increases the risk of serious cardiovascular side effects from oral contraceptive use. This risk increases with age and with heavy smoking (15 or more cigarettes per day) and is quite marked in women over 35 years of age. Women who use oral contraceptives should be strongly advised not to smoke.**

The use of oral contraceptives is associated with increased risk of several serious conditions including thromboembolism, stroke, myocardial infarction, hepatic adenoma, gallbladder disease, and hypertension. Practitioners prescribing oral contraceptives should be familiar with the following information relating to these risks.

1. **Thromboembolic Disorders and Other Vascular Problems:** An increased risk of thromboembolic and thrombotic disease associated with the use of oral contraceptives is well established. Studies have demonstrated an increased risk of fatal and nonfatal venous thromboembolism and stroke, both hemorrhagic and thrombotic.

**Cerebrovascular disorders:** In a collaborative study in women with and without predisposing causes, it was estimated that the risk of hemorrhagic stroke was 2.0 times greater in users than nonusers, and the risk of thrombotic stroke was 4.0 to 9.5 times greater.

**Myocardial infarction:** An increased risk of myocardial infarction associated with oral contraceptives has been reported confirming a previously suspected association. These studies found that the greater the number of underlying risk factors (cigarette smoking, hypertension, hypercholesterolemia, obesity, diabetes, history of preclampsic toxemia) for coronary artery disease, the higher the risk of developing myocardial infarction, regardless of whether the patient was an oral contraceptive user or not. Oral contraceptives, however, were found to be a clear additional risk factor.

It has been estimated that users who do not smoke (smoking is considered a major predisposing condition to myocardial infarction) are about twice as likely to have a fatal myocardial infarction as nonusers who do not smoke. Oral contraceptive users who are smokers have about a fivefold increased risk of fatal infarction compared to users who do not smoke, but about a tenfold to twelvefold increased risk compared to nonusers who do not smoke. The amount of smoking is also an important factor.

**Risk of dose:** In an analysis of data, British investigators concluded that the risk of thromboembolism including coronary thrombosis is directly related to the dose of estrogen used in oral contraceptives; however, the quantity of estrogen may not be the sole factor involved.

**Estimate of excess mortality from circulatory diseases:** The risk of diseases of the circulatory system is concentrated in older women, in those with a long duration of use, and in cigarette smokers.

A study of available data from a variety of sources concluded that the mortality associated with all methods of birth control is low and below that associated with childbirth, with the exception of oral contraceptives in women over 40 who smoke.

The risk of thromboembolic and thrombotic diseases associated with oral contraceptives increases with age after approximately age 30 and, for myocardial infarction, is further increased by hypertension, hypercholesterolemia, obesity, diabetes, or history of preclampsic toxemia, and especially by cigarette smoking.

The physician and the patient should be alert to the earliest manifestations of thromboembolic and thrombotic disorders. Should any occur or be suspected, the drug should be discontinued immediately.

A fourfold to sixfold increased risk of postsurgery thromboembolic complications has been reported in users. If feasible, oral contraceptives should be discontinued at least four weeks before surgery of a type associated with an increased risk of thromboembolism or prolonged immobilization.

2. **Ocular lesions:** Neuro-ocular lesions, such as optic neuritis or retinal thrombosis, have been associated with the use of oral contraceptives. Discontinue the oral contraceptive if there is unexplained sudden or gradual, partial, or complete loss of vision; onset of proptosis or diplopia; papilledema; or retinal vascular lesions.

3. **Carcinoma:** Long-term continuous administration of estrogen in certain animal species increases the frequency of carcinoma of the breast, cervix, vagina, and liver.

In humans, an increased risk of endometrial carcinoma associated with the prolonged use of exogenous estrogen in postmenopausal women has been reported. However, there is no evidence suggesting increased risk of endometrial cancer in users of conventional combination or progestogen-only oral contraceptives.

Studies found no evidence of increase in breast cancer in women taking oral contraceptives, however, an excess risk in users with documented benign breast disease was reported.

There is no confirmed evidence of an increased risk of cancer associated with oral contraceptives. Close clinical surveillance of users is, nevertheless, essential. In cases of undiagnosed persistent or recurrent abnormal vaginal bleeding, appropriate diagnostic measures should be taken to rule out malignancy. Women with a strong family history of breast cancer, or who have breast nodules, fibrocystic disease, or abnormal mammograms, should be monitored with particular care.

4. **Hepatic Tumors:** Benign hepatic adenomas have been found to be associated with oral contraceptives. Because hepatic adenomas may rupture and may cause death through intra-abdominal hemorrhage, they should be considered in women presenting abdominal pain and tenderness, abdominal mass, or shock.

A few cases of hepatocellular carcinoma have been reported in women taking oral contraceptives. The relationship of these drugs to this type of malignancy is not known at this time.

5. **Usage in or Immediately Preceding Pregnancy, Birth Defects in Offspring, and Malignancy in Female Offspring:** During early pregnancy, female sex hormones may seriously damage the offspring.

An increased risk of congenital anomalies, including heart defects and limb defects, has been reported with the use of oral contraceptives in pregnancy.

There is some evidence that triploidy and possibly other types of polyploidy are increased among abortuses from women who become pregnant soon after ceasing oral contraceptives.

Pregnancy should be ruled out before continuing an oral contraceptive in any patient who

has missed two consecutive menstrual periods. If the patient has not adhered to the schedule, the possibility of pregnancy should be considered at the time of the first missed period, and oral contraceptives should be withheld until pregnancy has been ruled out. If pregnancy is confirmed, the patient should be apprised of the potential risks to the fetus and the advisability of continuation of the pregnancy should be discussed.

Women who discontinue oral contraceptives with the intent of becoming pregnant should use an alternate form of contraception for a period of time before attempting to conceive.

Administration of progestogen-only or progestogen-estrogen combinations to induce withdrawal bleeding should not be used as a test of pregnancy.

6. **Gallbladder Disease:** Studies report an increased risk of surgically confirmed gallbladder disease in users of oral contraceptives.

7. **Carbohydrate and Lipid Metabolic Effects:** Because decreased glucose tolerance has been observed in a significant percentage of patients, prediabetic and diabetic patients should be carefully observed while receiving oral contraceptives.

An increase in triglycerides and total phospholipids has been observed.

8. **Elevated Blood Pressure:** An increase in blood pressure has been reported in patients receiving oral contraceptives. The prevalence in users increases with longer exposure. Age is also strongly correlated with development of hypertension. Women who previously have had hypertension during pregnancy may be more likely to develop elevation of blood pressure.

9. **Headache:** Onset or exacerbation of migraine or development of headache of a new pattern which is recurrent, persistent, or severe, requires discontinuation of oral contraceptives.

10. **Bleeding Irregularities:** Breakthrough bleeding, spotting, and amenorrhea are frequent reasons for patients discontinuing oral contraceptives. In breakthrough bleeding, nonfunctional causes should be borne in mind. In undiagnosed abnormal bleeding from the vagina, adequate diagnostic measures are indicated to rule out pregnancy or malignancy.

Women with a past history of oligomenorrhea or secondary amenorrhea, or young women without regular cycles should be advised that they may have a tendency to remain anovulatory or to become amenorrheic after discontinuation of oral contraceptives.

11. **Ectopic Pregnancy:** Ectopic as well as intrauterine pregnancy may occur in contraceptive failures.

12. **Breast Feeding:** Oral contraceptives may interfere with lactation. Furthermore, a small fraction of the hormonal agents in oral contraceptives has been identified in the milk of mothers receiving these drugs.

### PRECAUTIONS

1. A complete medical and family history should be taken prior to the initiation of oral contraceptives. The pretreatment and periodic physical examinations should include special reference to blood pressure, breasts, abdomen, and pelvic organs, including Papanicolaou smear and relevant laboratory tests. As a general rule, oral contraceptives should not be prescribed for longer than one year without another examination.

2. Preexisting uterine leiomyomata may increase in size.

3. Patients with a history of psychic depression should be carefully observed and the drug discontinued if depression recurs to a serious degree.

4. Oral contraceptives may cause fluid retention and should be prescribed with caution, and only with careful monitoring, in patients with conditions which might be aggravated.

5. Patients with a past history of jaundice during pregnancy have an increased risk of recurrence of jaundice. If jaundice develops, the medication should be discontinued.

6. Steroid hormones may be poorly metabolized and should be administered with caution in patients with impaired liver function.

7. Users may have disturbances in normal tryptophan metabolism, which may result in a relative pyridoxine deficiency.

8. Serum folate levels may be depressed.

9. The pathologist should be advised of oral contraceptive therapy when relevant specimens are submitted.

10. Certain endocrine and liver function tests and blood components may be affected.

(a) Increased sulfobromophthalein retention. (b) Increased prothrombin and factors VII, VIII, IX, and X; decreased antithrombin 3; increased norepinephrine-induced platelet aggregability. (c) Increased thyroid-binding globulin (TBG) leading to increased circulating total thyroid hormone. (d) Decreased pregnanediol excretion. (e) Reduced response to metoprolol test.

**Drug interactions:** Reduced efficacy and increased incidence of breakthrough bleeding have been associated with concomitant use of rifampin. A similar association has been suggested with barbiturates, phenylbutazone, phenytoin sodium, tetracycline, and ampicillin.

### ADVERSE REACTIONS

An increased risk of the following serious adverse reactions has been associated with oral contraceptives: thrombophlebitis; pulmonary embolism; coronary thrombosis; cerebral thrombosis; cerebral hemorrhage; hypertension; gallbladder disease; benign hepatomas; congenital anomalies.

There is evidence of an association between the following conditions and the use of oral contraceptives, although additional confirmatory studies are needed: mesenteric thrombosis, neuro-ocular lesions, eg, retinal thrombosis and optic neuritis.

The following adverse reactions have been reported in patients receiving oral contraceptives and are believed to be drug related: nausea and/or vomiting, usually the most common adverse reactions, occur in approximately 10% or less of patients during the first cycle. Other reactions, as a general rule, are seen much less frequently or only occasionally: gastrointestinal symptoms: breakthrough bleeding; spotting; change in menstrual flow; dysmenorrhea; amenorrhea during and after treatment; temporary infertility after discontinuance of treatment; edema; chloasma or melasma; breast changes; change in weight; change in cervical erosion and cervical secretion; possible diminution in lactation when given immediately postpartum; cholestatic jaundice; migraine; increase in size of uterine leiomyomata; rash (allergic); mental depression; reduced tolerance to carbohydrates; vaginal candidiasis; change in corneal curvature; intolerance to contact lenses.

The following adverse reactions have been reported and the association has been neither confirmed nor refuted: premenstrual-like syndrome; cataracts; changes in libido; chorea; changes in appetite; cystitis-like syndrome; headache; nervousness; dizziness; hirsutism; loss of scalp hair; erythema multiforme; erythema nodosum; hemorrhagic eruption; vaginitis; porphyria.

### Special Notes on Administration

Norlestrin (E) 1/50 (norethindrone acetate and ethinyl estradiol tablets, USP) and Norlestrin (B) 1/50—Menstruation usually begins two or three days, but may begin as late as the fourth or fifth day, after the brown ferrous fumarate or white inert tablets have been started.

Norlestrin (A) 1/50—Menstruation usually begins two or three days, but may begin as late as the fourth or fifth day, after discontinuing medication.

After several months on treatment, bleeding may be reduced to a point of virtual absence; reduced flow may be a result of medication and not indicative of pregnancy.

### HOW SUPPLIED

Norlestrin (E) 1/50 is available in minicompacts each containing 21 yellow tablets and 7 brown tablets. Each yellow tablet contains 1 mg of norethindrone acetate and 50 mcg of ethinyl estradiol. Each brown tablet contains 75 mg of ferrous fumarate. Available in packages of five minicompacts and packages of five refills.

Norlestrin (B) 1/50 is available in minicompacts each containing 21 yellow tablets and 7 white inert tablets. Each yellow tablet contains 1 mg of norethindrone acetate and 50 mcg of ethinyl estradiol. Available in packages of five minicompacts and in packages of five refills.

Norlestrin (A) 1/50 is available in minicompacts each containing 21 yellow tablets. Each yellow tablet contains 1 mg of norethindrone acetate and 50 mcg of ethinyl estradiol. Available in packages of five minicompacts and packages of five refills.

0904G010

**Direct Medical Inquiries to:** Parke-Davis, Div Warner-Lambert Inc, 201 Tabor Road, Morris Plains, NJ 07950 Attn: Medical Affairs Department

**PARKE-DAVIS**

Div Warner-Lambert Inc  
Santurce, PR 00911

PD-JA-0270-2-P(12-80)



# Amniotic fluid prolactin levels and lecithin/sphingomyelin ratios during the third trimester of human gestation

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CHUNG H. WU, M.D.

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Prolactin levels in amniotic fluid during the third trimester of human pregnancy showed a wide range of values. There was no apparent correlation between amniotic fluid prolactin concentrations (AF PRL) and lecithin/sphingomyelin (L/S) ratios nor was there a prolactin concentration associated with a mature L/S ratio. There was no correlation between AF PRL concentration and estimated gestational age or neonatal birth weight. In patients with diabetes mellitus (White Classes A to C), AF PRL were higher prior to 37 weeks, or when the L/S ratio was less than 2, than after 37 weeks, or when the L/S ratio was greater than 2. (AM. J. OBSTET. GYNECOL. 139:435, 1981.)

FETAL SERUM PROLACTIN (PRL) has been implicated in the functional maturation of the fetal lung and in augmented surfactant production by type II pneumocytes. However, a causal relationship between elevated fetal serum PRL concentrations and fetal lung maturation has not been unequivocally demonstrated.<sup>1-10</sup> While several animal and human studies have suggested that fetal PRL may be involved in fetal adrenal maturation and lung surfactant production, others have not supported these conclusions.

In contrast, the role of amniotic fluid (AF) PRL in the process of fetal lung maturation has not been adequately examined.<sup>11-17</sup> In a recent report, the AF PRL concentration has been correlated with AF lecithin/sphingomyelin (L/S) ratios and fetal lung stability characteristics in rhesus monkeys.<sup>18</sup> In human pregnancies, however, AF PRL did not correlate with L/S ratio.<sup>19</sup> This study did not include patients prior to 36

weeks' gestation nor did it appear to include a significant number of complicated pregnancies. The retrospective investigation reported here was thus undertaken to answer the question whether AF PRL is involved in maturational processes in the human fetal lung by examining the relationship between the AF PRL concentration and the AF L/S ratio as well as the estimated gestational age in the last trimester of human pregnancies.

## Material and methods

One hundred AF specimens were obtained by transabdominal amniocentesis in patients at 28 to 40 weeks' gestation. The primary indication for amniocentesis was the need for determination of an L/S ratio. AF free of blood or meconium was obtained from normal patients (39 samples) as well as from patients with diabetes mellitus (White Classes A to C, 15 samples), hypertension (14 samples), Rh isoimmunization (14 samples), and intrauterine fetal growth retardation (7 samples). In 12 of these cases, serial AF samples were available for examination. An additional 11 AF specimens were obtained from patients in labor. Forty-nine AF specimens had been obtained within 1 week of delivery. The AF fluid was first centrifuged at 3,000 rpm for 10 minutes. A portion of the supernatant was used for L/S determination. After methanol/chloroform extraction and acetone precipitation, lecithin and sphin-

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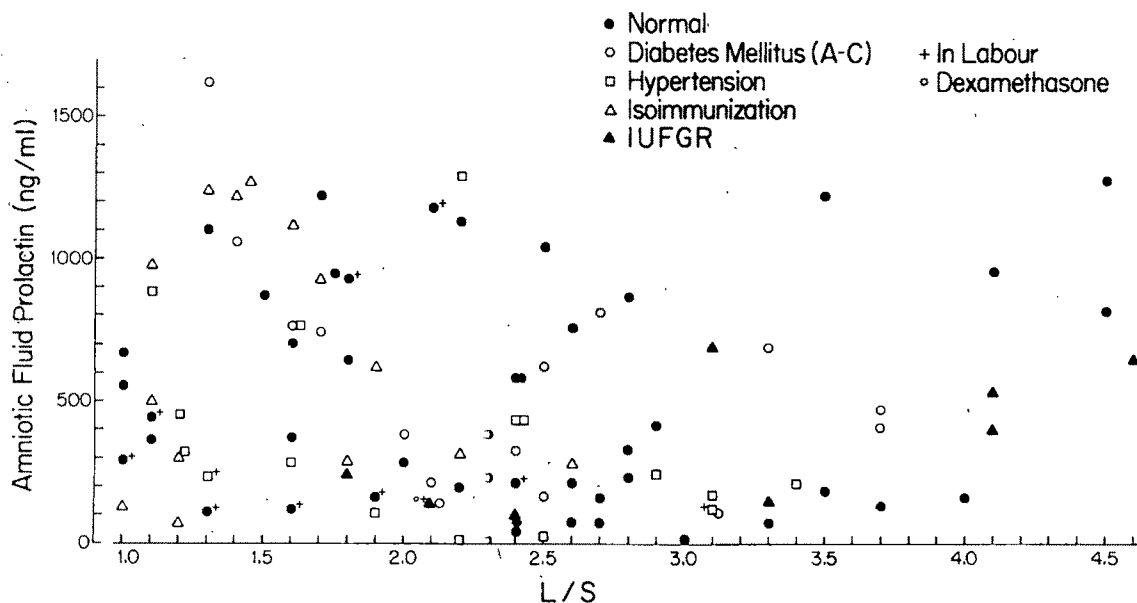


Fig. 1. Scattergram of AF PRL concentrations versus L/S ratios.

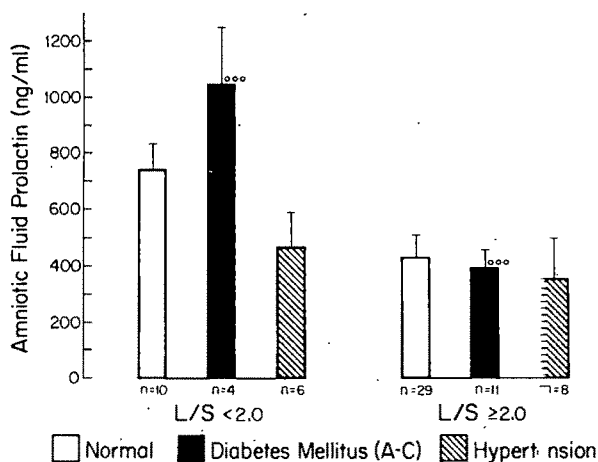


Fig. 2. Mean  $\pm$  SEM of AF PRL concentration for normal, diabetic, and hypertensive patients grouped according to L/S ratios.

gomyelin fractions were separated by thin-layer chromatography. L/S ratios were calculated by planimetry.\* The remaining AF was stored at  $-20^{\circ}\text{C}$  until it was assayed for human PRL. The AF PRL concentration was determined by a homologous double-antibody radioimmunoassay† modified after the method of Sinha and associates.<sup>20</sup> The lowest concentration measurable by this procedure was 1.67 ng/ml. The intra-assay co-

\*Chromato/O/Screen L/S ratio Analysis Kit, Supelco, Inc., Bellefonte, Pennsylvania

†hPRL, RIA Kit, Sero Laboratories, Braintree, Massachusetts.

efficient of variation was 8.1% while the interassay coefficient of variation was 12.4%. Gestational age was estimated by a combination of clinical and ultrasonographic criteria. Data were analyzed by single and multiple regression analyses as well as by analysis of variance. A P value less than 0.01 was accepted as significant.

### Results

**AF PRL versus L/S.** Fig. 1 shows the scattergram of AF PRL concentrations plotted as a function of L/S ratios in all patients. There were extreme degrees of variability in the PRL concentrations among samples of fluid obtained from different patients of corresponding L/S ratios. Single as well as multiple regression analyses of all data failed to reveal any significant correlation between these two variables. The data were then grouped by patient category as a function of L/S ratios. Because of insufficient numbers of patients in some cells, further analysis was limited to only three categories. Fig. 2 shows the mean and standard error of the mean AF PRL concentrations in normal patients as well as in diabetic and hypertensive patients.

AF PRL concentrations in patients with diabetes and L/S ratios  $<2$  were higher than in those with L/S ratios  $>2$  ( $F = 9.74$ ,  $P < 0.01$ ). Intragroup comparisons did not reveal any significant differences between mean AF PRL levels in the three patient categories.

**AF PRL versus estimated gestational age.** Fig. 3 shows the scattergram of the AF PRL concentration versus estimated gestational age in all patients. Single



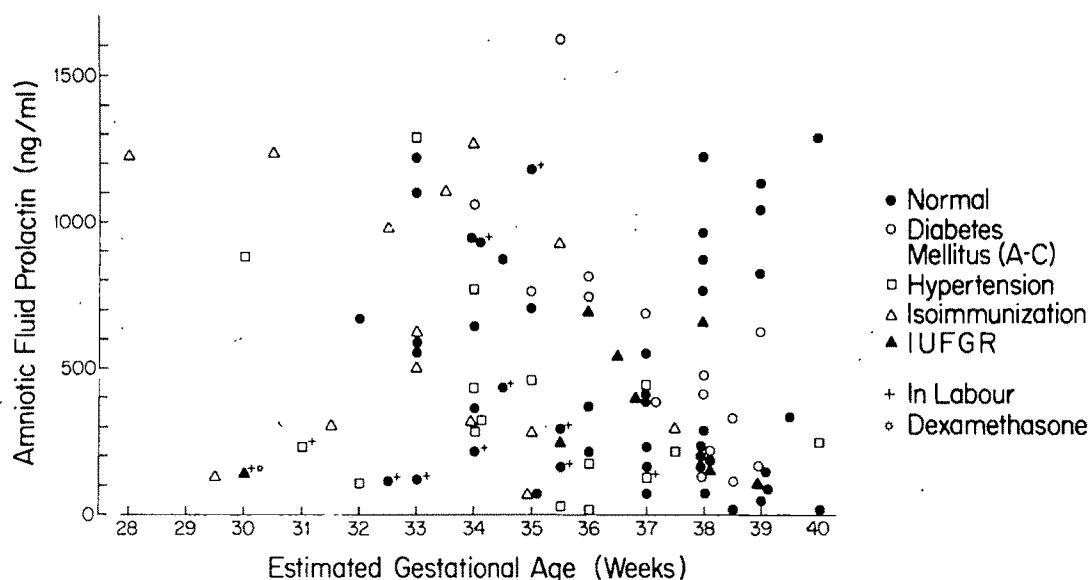


Fig. 3. Scattergram of AF PRL concentrations versus estimated gestational age.

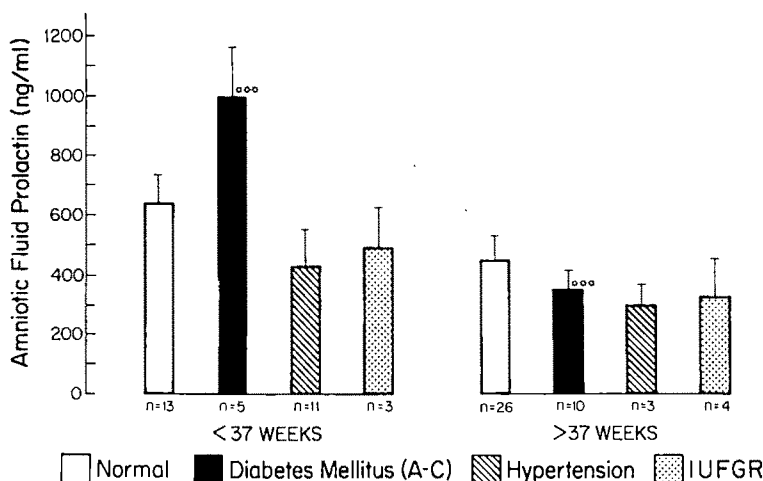


Fig. 4. Mean  $\pm$  SEM of AF PRL concentration from uncomplicated pregnancies and pregnancies complicated by diabetes, hypertension, and intrauterine fetal growth retardation grouped according to gestational age.

as well as multiple regression analyses of all data failed to reveal any significant correlation between these two variables. A wide scatter of values was again noted. The AF PRL levels were then grouped by patient category as a function of estimated gestational age. Because of insufficient numbers of patients in some cells, analysis was limited to four patient categories. Fig. 4 shows the mean and standard error of the mean of AF PRL concentrations in normal, diabetic, and hypertensive patients as well as patients with intrauterine fetal growth retardation. AF PRL levels in patients with diabetes and an estimated gestational age of less than 37 weeks

were higher than in those with an estimated gestational age of greater than 37 weeks ( $F = 10.98$ ,  $P < 0.01$ ). Intragroup comparisons did not reveal any significant differences between mean AF PRL levels in the four patient categories.

Fig. 5 shows the scattergram of AF PRL concentrations versus neonatal birth weight in cases where amniocentesis had been performed within 1 week of delivery. Regression analysis failed to reveal any significant correlation between these two variables. When normal patients and patients with diabetes were compared, no significant difference could be found in the

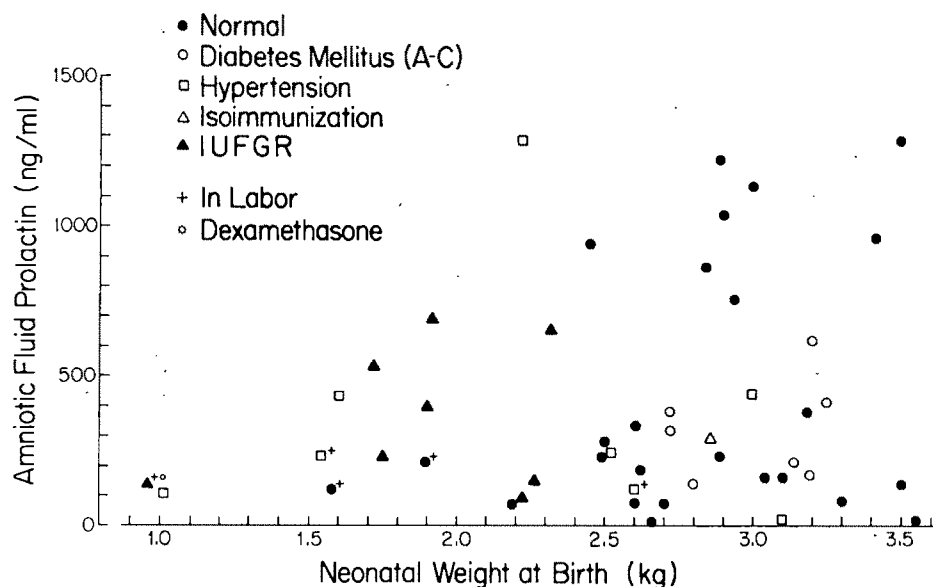


Fig. 5. Scattergram of AF PRL concentrations versus neonatal birth weight (AF was obtained within 1 week of delivery).

mean PRL concentrations and the mean weight at birth. Finally, examination of serial samples in 12 patients failed to reveal any consistent trend.

#### Comment

Elevated fetal serum PRL levels have been associated with functional maturation of the fetal lung and augmented surfactant production by type II pneumocytes. This hypothesis is based on the observation that the rise in fetal PRL levels during the latter part of pregnancy coincides with an increase of fetal adrenal weight<sup>1</sup> and precedes the onset of augmented cortisol and phosphatidylcholine production by the fetal adrenal and fetal lung, respectively.<sup>2</sup> However, the evidence that appears to support this hypothesis is not conclusive. First, preliminary *in vivo* studies have failed to demonstrate that PRL can function as an adrenocorticotrophic hormone.<sup>2-4</sup> Second, the evidence from investigations with fetal sheep and rabbits concerning a direct stimulatory influence of PRL on surfactant production is conflicting.<sup>2, 5, 6</sup> Third, the reported association between lower umbilical cord blood PRL levels and an increased incidence of respiratory distress syndrome that was suggested in two studies<sup>7, 8</sup> has not been confirmed by other investigators.<sup>9, 10</sup>

AF PRL is identical to that found in the pituitary and serum.<sup>21</sup> However, its origin and functions are not well understood. Decidual tissue appears to be the most likely source of AF PRL. *In vitro* systems, this tissue has been found capable of both release and *de novo* synthesis of PRL.<sup>22</sup> PRL originating from the mater-

nal<sup>23</sup> and/or fetal<sup>2, 24</sup> pituitary also may contribute to the AF PRL levels.

AF PRL may function primarily as a regulator of AF production and osmolality.<sup>25-27</sup> In contrast to fetal plasma PRL, AF PRL has not been implicated in fetal growth, adrenal function, or lung maturation. However, the observation by Johnson and associates<sup>18</sup> that AF PRL may play a role in fetal rhesus monkey lung maturation is intriguing. It has been reported that PRL receptors are present in the lung parenchyma of these fetuses.<sup>28</sup> There is very little information regarding a possible relationship between L/S ratio<sup>19</sup> or fetal lung stability characteristics and AF PRL in the last trimester of human pregnancy. Thus, we elected to examine this question with respect to AF L/S changes and estimated gestational age.

It is readily apparent that in the third trimester of human pregnancy there are extreme degrees of variability in the concentrations of AF PRL. Intrinsic variability in its production by decidua<sup>29</sup> and/or AF volume changes may contribute to this wide range of values. We have not performed measurements of AF volume. However, it is doubtful whether the AF PRL:constant denominator ratio can significantly affect the data.

We could not demonstrate the existence of a relationship between increasing L/S ratios and AF PRL concentrations. Moreover, there was no AF PRL level below (or above) which the L/S ratio would be greater (or less) than 2. These observations would seem to indicate that AF PRL is not directly involved in maturational processes in the human fetal lung. This is at vari-



ance with previously reported results from rhesus monkeys.<sup>18</sup> However, interspecies differences might have contributed to our differing findings.

Our findings concerning AF PRL concentrations during various stages of pregnancy from 28 to 30 weeks until term seem to indicate that a well-defined and consistent pattern of decreasing concentrations with advancing gestational age is not readily apparent. Although other investigators<sup>15, 17</sup> have reported declining levels, the wide range of values obtained for each gestational age has not allowed rigorous statistical evaluation.<sup>11-17</sup> Moreover, serial samples from some patients do not reveal any consistent trends.

Our observations concerning AF PRL concentrations in patients with diabetes mellitus, although statistically significant, are derived from a small number of pa-

tients. The absence of macrosomic infants (Fig. 5) and clinically overt polyhydramnios in our diabetic patients does not allow us to attribute our observations to a greater degree of dilution of AF PRL.

Because we did not measure AF osmolality, we cannot comment on a possible relationship between AF PRL and osmolality. Finally, to what extent, if any, local factors (for example, PRL synthesis by decidua) can account for our observations in diabetic patients is not known.

Obviously, our results do not exclude the possibility that fetal serum PRL is involved either directly or indirectly in conjunction with other hormones in inducing surfactant production in the lung and thereby preventing respiratory distress syndrome.<sup>30</sup> Further investigation into this matter is warranted.

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# Fetal metabolic response to endogenous insulin release

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The fetal metabolic response to tolbutamide-induced insulin release was evaluated in nine chronically catheterized fetal lambs. Tolbutamide produced an elevation in fetal insulin that persisted throughout the 1-hour experimental period (peak insulin concentration achieved was  $76.1 \pm 9.5$   $\mu\text{U/ml}$ ). Fetal hyperinsulinemia was associated with a fall by 30 minutes in both fetal arterial and umbilical venous glucose concentrations as well as an increase in the umbilical glucose venous-arterial difference. This increase was associated with an elevation of fetal glucose uptake  $65.6\% \pm 29.6\%$  above control at 30 minutes after infusion. Fetal arterial oxygen content fell after tolbutamide administration and was accompanied by an increase in the umbilical venous-arterial oxygen content difference ( $13.0\% \pm 5.0\%$  above the basal level.) Despite the increased venous-arterial difference, however, no change in umbilical oxygen consumption was noted. Isolated endogenous fetal insulin release is thus associated with a relatively rapid increase in umbilical glucose uptake as well as concomitant fetal hypoglycemia. The etiology of the mild fetal hypoxemia produced is unclear. The alterations in fetal glucose uptake in the ovine fetus after tolbutamide mimic those changes occurring after fetal exogenous insulin infusion. These alterations strongly suggest a significant role for insulin in the modulation of fetal carbohydrate metabolism and perhaps oxygen utilization. (AM. J. OBSTET. GYNECOL. 139:441, 1981.)

INSULIN is a well-known anabolic hormone whose role concerning fetal growth is unclear. In vivo experiments have demonstrated significant release of fetal insulin in response to appropriate stimuli such as glucose in a number of species.<sup>1-3</sup> However, glucose-induced endogenous fetal insulin release has not been demonstrated in vivo to cause any significant immediate metabolic response. Unfortunately, a stimulus such as glucose infusion may function not only to stimulate insulin secretion but also to obscure the metabolic response to endogenous insulin release. Exogenous insulin infused into the fetus causes hypoglycemia<sup>4</sup> and

recent evidence suggests that this hypoglycemia is due to increased fetal glucose consumption.<sup>5, 6</sup> Prolonged fetal insulin infusion may increase fetal oxygen consumption as well.<sup>5</sup>

The sulfonylurea tolbutamide releases stored insulin from the pancreatic  $\beta$ -cell and has been used in the fetal sheep<sup>7</sup> and monkey<sup>2</sup> to induce a transient endogenous hyperinsulinism. Philipps and co-workers<sup>7</sup> have shown a consistent hypoglycemic response to tolbutamide-induced insulin release in the fetal lamb and have suggested that this response may be the result of increased fetal glucose utilization. Therefore, tolbutamide was injected into chronically catheterized fetal lambs to explore further and quantitate possible alterations in glucose and oxygen consumption secondary to endogenous insulin release.

## Material and methods

**Surgical preparation.** Nine pregnant ewes were studied between 110 and 145 days' gestational age (term pregnancy in sheep is 147 days). All ewes were given intravenous sodium pentobarbital and a spinal anesthetic (15 mg of Pontocaine) preoperatively. Polyvinyl catheters were placed in the umbilical vein, fetal

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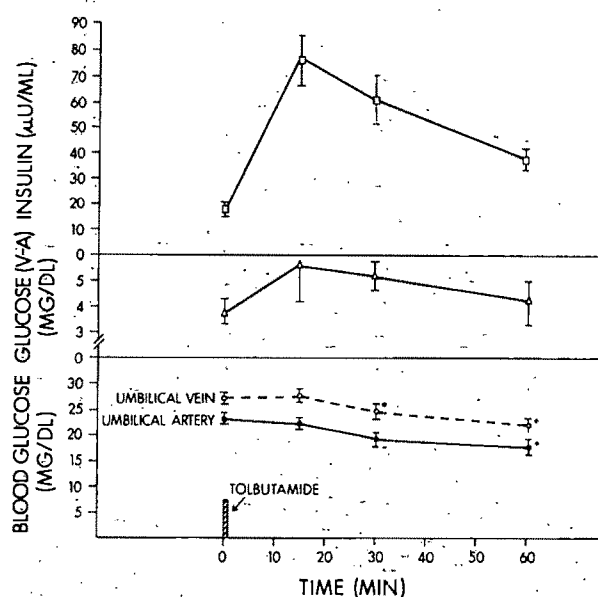


Fig. 1. Fetal plasma insulin, umbilical venous and arterial glucose; and glucose venous-arterial differences in response to tolbutamide infusion. \* $p < 0.01$ . \* $p < 0.001$ .

pedal artery, and two fetal pedal veins as well as the maternal femoral artery for purposes of sampling and infusion. Catheters were then tunneled subcutaneously to the mother's flank. Postoperative care was performed as previously described<sup>8</sup> and all animals were allowed at least 4 postoperative days before experiments were begun.

**Experimental design.** Tolbutamide (Orinase, The Upjohn Co., Kalamazoo, Michigan) was infused into a fetal venous catheter on 18 occasions in the nine experimental animals (Table I). A dose of 100 to 200 mg/kg of estimated fetal weight was injected in 10 ml of normal saline by hand over 60 seconds. Since in a previously reported study<sup>7</sup> no lasting effects of acute tolbutamide injection upon insulin secretion or glucose metabolism were noted, serial infusions were performed in some animals. However, a 48- to 72-hour recovery period separated exposure to tolbutamide in these animals. Two animals received placebo injections of 10 ml of normal saline instead of tolbutamide.

During a 1-hour control period, fetal blood samples were drawn four to six times for determination of umbilical venous and arterial whole-blood glucose and oxygen content as well as arterial immunoreactive insulin concentration. Fetal arterial pH,  $P_{CO_2}$ , and  $P_{O_2}$  were obtained during the control and experimental periods and maternal arterial glucose and insulin were also assessed. During the control period, umbilical blood flow determinations were made by means of the antipyrine steady-state diffusion technique.<sup>9</sup> A separate

Table I. Summary of animal preparations utilized

| Animal No. | Gestational age on day of surgery (days) | Gestational age at injection (days) | Singleton (S) or twin (T) | Pregnancy outcome                       |
|------------|------------------------------------------|-------------------------------------|---------------------------|-----------------------------------------|
| 1          | 121                                      | 133                                 | S                         | Term, live-born                         |
| 2          | 123                                      | 128<br>131<br>141                   | S                         | Term, live-born                         |
| 3          | 124                                      | 128<br>130                          | T                         | Fetal death 10 days after last infusion |
| 4          | 105                                      | 112                                 | T                         | Fetal death 14 days after infusion      |
| 5          | 131                                      | 144                                 | S                         | Term, live-born                         |
| 6          | 121                                      | 126<br>134                          | S                         | Term, live-born                         |
| 7          | 128                                      | 132                                 | T                         | Fetal death 5 days after infusion       |
| 8          | 124                                      | 128<br>135<br>137                   | S                         | Term live-born                          |
| 9          | 123                                      | 127<br>130<br>134<br>137            | S                         | Term, live-born                         |

fetal venous catheter was used for antipyrine infusion. Glucose consumption ( $Q_{glu}$ ) was calculated from the Fick principle:

$$Q_{glu} \text{ (mg/kg} \cdot \text{min)} = \frac{\text{blood glucose venous-arterial difference (mg/dl)} \times \text{umbilical blood flow (ml/kg} \cdot \text{min)}}{\text{blood glucose venous-arterial difference (mg/dl)}}$$

Fetal weight at the time of the experiment was estimated by extrapolating from the known birth weight with the use of fetal lamb growth charts for either twin or singleton pregnancies. Similar calculations were made for assessment of fetal oxygen consumption ( $Q_{O_2}$ ).

During the experimental period after tolbutamide infusion, simultaneous arterial and umbilical venous samples were drawn at 15, 30, and 60 minutes to assess whole-blood glucose and oxygen contents as well as arterial insulin and umbilical blood flow.

**Biochemical studies.** Blood glucose was assayed by means of a glucose oxidase technique,<sup>10</sup> oxygen content was determined with the Lex-O<sub>2</sub> Coni (Waltham, Massachusetts),<sup>11</sup> and antipyrine concentration was measured from whole blood with a Technicon Auto-Analyzer. Radioimmunoassayable insulin was measured by means of a modification<sup>3</sup> of the method of Morgan and Lazarow.<sup>12</sup> Ovine insulin standards were supplied by Mary Root, Eli Lilly & Co., Indianapolis, Indiana.

**Statistical methods.** All results are expressed as the mean  $\pm$  1 SEM. Statistical significance was assessed by



**Table II.** Umbilical glucose and oxygen consumptions and UBF after tolbutamide injection

|         | $\dot{Q}_{glu}$<br>(ml/kg · min) | $\dot{Q}_{O_2}$<br>(ml/kg · min) | UBF<br>(ml/kg · min) |
|---------|----------------------------------|----------------------------------|----------------------|
| Control | $6.70 \pm 0.79$<br>(16)          | $8.01 \pm 0.37$<br>(16)          | $182 \pm 9$<br>(16)  |
| 15 min  | $8.98 \pm 2.10$<br>(13)          | $8.48 \pm 2.17$<br>(13)          | $174 \pm 12$<br>(13) |
| 30 min  | $8.89 \pm 0.96$<br>(15)          | $7.91 \pm 0.38$<br>(16)          | $170 \pm 9$<br>(16)  |
| 60 min  | $6.67 \pm 1.41$<br>(15)          | $8.21 \pm 0.68$<br>(15)          | $167 \pm 12$<br>(16) |

means of the paired Student's *t* test. Linear regression analysis was performed by the least-squares method.

### Results

Table I presents the gestational age at the time of surgery and injection for the nine animals studied and the pregnancy outcome. As can be seen, six of nine ewes delivered healthy term lambs. None of the three fetal deaths was temporally related to tolbutamide injection and no significant changes in fetal pH,  $PO_2$  or  $PCO_2$  were demonstrated after tolbutamide injection. Fig. 1 demonstrates the data related to fetal insulin and glucose concentration. No effect of gestational age upon insulin response or changes in glucose concentration could be detected and so the data were pooled. Control insulin concentration rose from  $18.1 \pm 2.4$   $\mu$ U/ml to a maximum of  $76.1 \pm 9.5$   $\mu$ U/ml at 15 minutes after tolbutamide injection ( $p < 0.001$ ) and then gradually fell. Umbilical venous and arterial glucose concentrations were unchanged until 30 minutes after injection, when they fell significantly ( $p < 0.01$ ) below control values. A nadir was reached at 60 minutes for both venous and arterial glucose concentrations, which fell  $5.9 \pm 1.3$  and  $6.5 \pm 1.3$  mg/dl below baseline, respectively. However, no relation could be found between the fall in glucose and peak insulin concentration achieved. No significant changes occurred in maternal arterial glucose or insulin concentrations after fetal tolbutamide injection.

The mean venous-arterial glucose difference (Fig. 1) was above the basal value of  $3.62 \pm 0.36$  mg/dl at all experimental points but, because of interanimal variability, none was significantly elevated ( $p < 0.06$  at 30 minutes). In order to decrease this variability, results were expressed as the percentage of change from the basal venous-arterial difference. A significant elevation ( $172\% \pm 30\%$  above baseline,  $p < 0.05$ ) was noted at 30 minutes.

Fig. 2 shows the same series of experiments as related to changes in oxygen content. Umbilical venous

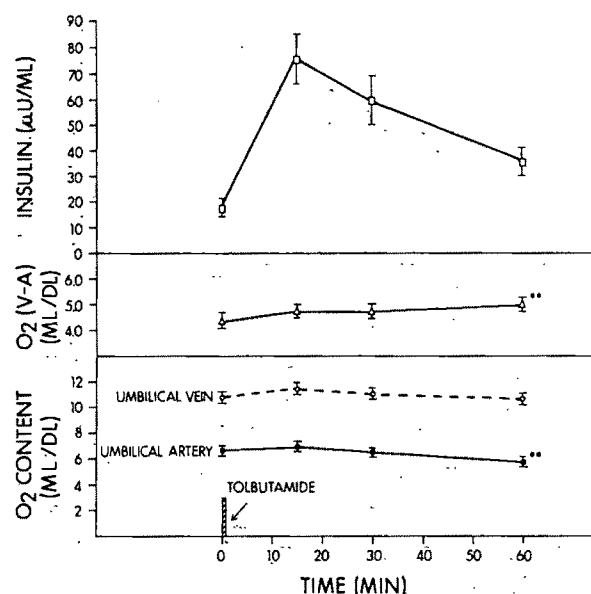


**Fig. 2.** Fetal umbilical venous and arterial oxygen content and oxygen venous-arterial differences in response to tolbutamide infusion. \* $p < 0.01$ .

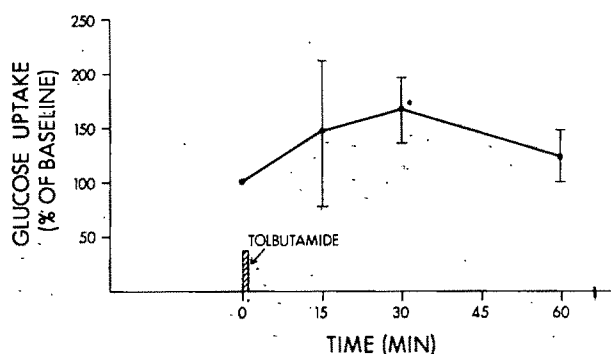


**Fig. 3.** Fetal umbilical glucose uptake after tolbutamide injection. \* $p < 0.05$ .

oxygen content did not change significantly during the experiment. However, fetal arterial oxygen content did fall significantly by 60 minutes after infusion ( $\Delta = -0.71 \pm 0.20$  ml/dl,  $p < 0.01$ ). Venous-arterial oxygen content difference as depicted in Fig. 2, was also increased from the basal value of  $4.43 \pm 0.16$  ml/dl at 60 minutes after infusion ( $\Delta = +0.67 \pm 0.18$  ml/dl,  $p < 0.05$ ). If these results are expressed as the percentage of change from baseline as before, the change in venous-arterial oxygen content difference remains significant ( $13.0\% \pm 5.0\%$  above baseline,  $p < 0.05$ ).

Basal glucose and oxygen consumptions, as well as basal umbilical blood flow (UBF) data, are presented in Table II. No statistically significant changes in umbilical blood flow were evident after tolbutamide injection.

Mean glucose consumptions at 15 and 30 minutes after tolbutamide injection were above control values but were not significantly elevated. As before, however, if expressed as a percentage of the control value, glucose consumption was significantly elevated at 30 minutes after injection (Fig. 3,  $\Delta = +65.6\% \pm 29.6\%$ ,  $p < 0.05$ ). Fetal oxygen consumption (Table II) was above baseline at two of the three experimental points but the differences were not statistically significant, nor was a significant change noted when results were expressed as the percentage of change from baseline.

Two placebo injections with saline produced no change from control in any of the parameters measured. Likewise, two injections of tolbutamide in separate animals produced no significant insulin response for unknown reasons. In neither of these animals was there any change in glucose or oxygen content and consumption or change in UBF.

#### Comment

Tolbutamide-induced insulin release in the fetal sheep was associated with a fall in both fetal arterial and umbilical venous glucose to approximately 75% of the basal concentration, similar to values obtained in our previously published report. A latent period of 15 to 30 minutes was evident prior to this response and, despite a large amount of interanimal variability, significant increases in the percentage of change from basal venous-arterial glucose difference and umbilical glucose uptake were noted at 30 minutes after infusion. As in our prior study,<sup>7</sup> advancing gestational age in the last month of pregnancy was not associated with detected alterations in any of the variables measured. The interanimal variability noted is most probably related to a variety of factors, particularly the length and magnitude of insulin response to tolbutamide. Biological variation as well as inaccuracies in fetal weight estimation and, thus, dosage standardization may have been responsible.

Prior indirect evidence in fetal rat and monkey preparations has suggested that exogenous insulin causes both increased fetal glucose uptake<sup>13</sup> and glycogen synthesis<sup>14</sup> and an increase in nitrogen and lipid content of the fetus.<sup>15</sup> Colwill and co-workers,<sup>4</sup> in an *in vivo* chronically catheterized sheep preparation, demonstrated that pharmacologic doses of insulin injected into the fetus caused both fetal hypoglycemia and an associated increase in the umbilical venous-arterial glucose difference 1 hour after injection. Simmons and associates,<sup>6</sup> also using pharmacologic insulin dosages, have more recently demonstrated that fetal insulin infusion can cause as much as a 50% increase in fetal glucose uptake after 1½ hours of administration. More

physiologic infusions of insulin have also been associated with fetal hypoglycemia, increased venous-arterial glucose difference, and an increased glucose/oxygen quotient when infused over several days.<sup>5</sup>

Only limited data are available, however, regarding the immediate metabolic response to endogenous insulin release in fetal life. Our data conflict with those of Mintz and co-workers,<sup>2</sup> who found that tolbutamide injections into fetal monkeys at 90% of term caused a release of insulin but had no demonstrable effect on fetal glucose levels. However, only three experiments were performed and the last sample time was 30 minutes after injection. Interspecies variation in responsiveness to tolbutamide plus differences in dosage may also have contributed to this discrepancy. Our investigation demonstrated an increased fetal glucose uptake within 30 minutes after endogenous insulin release, which is consistent with a possible role for insulin in the control of fetal carbohydrate metabolism. This lag time between insulin secretion and maximal metabolic effect is strikingly similar to that found by Ganda and co-workers<sup>16</sup> in human adult volunteers and underlines further the probable causal relationship between insulin release and glucose uptake.

The mechanism responsible for the insulin-induced increase in fetal glucose uptake is unclear. Although enhanced fetal carcass and hepatic uptake are most likely, we cannot exclude the possibility that insulin-induced suppression of fetal hepatic glucose output, as shown by Sacca and associates<sup>17</sup> in the adult human, may contribute to the mechanism for the fetal hypoglycemia seen after tolbutamide. In either case, a net increase in fetal glucose uptake would be observed. It is possible that continued fetal-maternal glucose transfer, as demonstrated in the sheep by Anand and co-workers,<sup>18</sup> could have contributed to the fetal hypoglycemia, although this appears unlikely. There is presently no evidence to suggest that fetal hyperinsulinism alters placental glucose clearance. Furthermore, in the presence of enhanced fetal-maternal glucose transfer, the umbilical venous-arterial glucose difference (which increased in our preparations) would have been expected to fall.

Tolbutamide-induced insulin release was associated with a significant fall in fetal arterial oxygen content and a concomitant increase in mean venous-arterial oxygen content difference. This relationship is similar to that found by Carson and co-workers,<sup>5</sup> who utilized exogenous insulin, although sampling was not begun until 4 hours of infusion in that study. Insulin has been shown to enhance mitochondrial oxidative phosphorylation in isolated mammalian liver preparations<sup>19</sup> and might theoretically increase both the rate of substrate



oxidation and oxygen consumption.<sup>20</sup> However, although the arterial oxygen content fell in our experiments, a statistically significant increase in oxygen consumption could not be demonstrated, making the role of acute hyperinsulinemia in regulation of oxygen consumption unclear.

These experiments do not address the possibility that tolbutamide itself might be primarily responsible for the results seen. Although long-term administration of the sulfonylureas has been associated with a variety of peripheral metabolic effects,<sup>21, 22</sup> it is clear that after pancreatectomy the acute administration of sulfonylureas does not lower blood sugar.<sup>23</sup> Furthermore, it is significant that two fetal lambs who failed to respond to tolbutamide injection with elevation of plasma insulin concentration also failed to have any significant alterations in umbilical glucose or oxygen uptake. Last, our results mimic both the hypoglycemia and the increase

in fetal glucose consumption as well as the fall in arterial oxygen content seen after exogenous insulin administration, suggesting that fetal insulin release is indeed the causative factor.

In summary, the hypoglycemia seen after endogenous fetal insulin release is associated with elevation of the umbilical venous-arterial glucose difference as well as enhancement of fetal glucose uptake within 15 minutes of the peak fetal insulin response. This information suggests that control of fetal carbohydrate metabolism not only is linked to fetal insulin release but also may be very responsive to rapid alterations in circulating insulin. Although a mild fetal hypoxemia occurred after tolbutamide, mimicking the response to exogenous insulin infusion, we were unable to demonstrate a significant increase in umbilical oxygen consumption.

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# Significance of the sinusoidal fetal heart rate pattern

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The sinusoidal fetal heart rate pattern has been reported to be an indication of fetal compromise. In this review of 31 cases, drawn from a total of 559 monitored cases of normal and at-risk women, only one resulted in poor outcome, and this was attributable to a difficult breech delivery. An association with administration of analgesics is confirmed. The sinusoidal fetal heart rate pattern does not appear to be uniformly associated with fetal distress. Its presence should be an indication for meticulous assessment of other electrocardiographic, biochemical, or clinical evidence of fetal status. (AM. J. OBSTET. GYNECOL. 139:446, 1981.)

THE CLINICAL OCCURRENCE of a "sinusoidal" fetal heart rate pattern has been documented in a series of reports.<sup>1-22</sup> The pattern was described by Kubli and associates,<sup>1</sup> who identified a sinusoidal waveform with a frequency of 2 to 5 cycles per minute (cpm) in tracings from nine of twelve fetuses who died in utero. They believed this to be a "typical sign of severe fetal compromise." Manseau and associates<sup>2</sup> also recognized a sinusoidal pattern characterized by an amplitude of 5 to 15 beats per minute (bpm), a wavelength of 15 to 30 seconds, and a fixed, regular sinusoidal rhythmicity. Of 11 reported cases, nine were complicated by rhesus isoimmunization and there were seven deaths (six in rhesus-sensitized fetuses). In two tracings, the pattern was evanescent and the outcome was good. The pathophysiology of a sinusoidal pattern was unclear to both groups, although the association with rhesus sensitization was remarkable.

Baskett and Koh<sup>3</sup> described a pattern with an

amplitude of 20 to 25 bpm and a frequency of 2 to 3 cpm in an infant who died neonatally. They also reported several anecdotal cases with good neonatal outcome. The large series of Rochard and associates<sup>4</sup> (using 2 to 5 cpm and 5 to 10 bpm as a definition of the sinusoidal waveform) reported 20 sinusoidal patterns in nonstress test monitoring of fetuses with erythroblastosis fetalis whose outcome was generally poor. However, Mueller-Heubach and associates<sup>5</sup> and Hatjis and associates<sup>10</sup> managed fetuses with erythroblastosis fetalis and repetitive sinusoidal patterns both before and after intrauterine transfusion with good eventual outcome. Rochard and associates<sup>5</sup> ascribed the pattern to "a virtual absence of central nervous system control over the heart rate." The high-risk status of many of the other reported cases (postdates,<sup>4, 7</sup> severe diabetes,<sup>1, 7</sup> pre-eclampsia,<sup>1, 11, 13, 14</sup> and amnionitis<sup>21</sup>) makes evaluation of the significance of this pattern, with respect to perinatal outcome, even more difficult, especially since the outcome was not uniformly poor.<sup>2, 3, 9, 12, 14-16, 21</sup>

Gray and associates<sup>10</sup> showed an association of the pattern with the administration of the narcotic analgesic alphaprodine and all their study infants did well.

The proposed clinical management based on these conflicting case reports has varied from simple continued observation to extensive biochemical assessment of the fetus, and even to immediate delivery. The purpose of this study was to ascertain the incidence of the sinusoidal fetal heart rate pattern in a group of monitored fetuses and to make clinical recommendations, based on the observed outcome.

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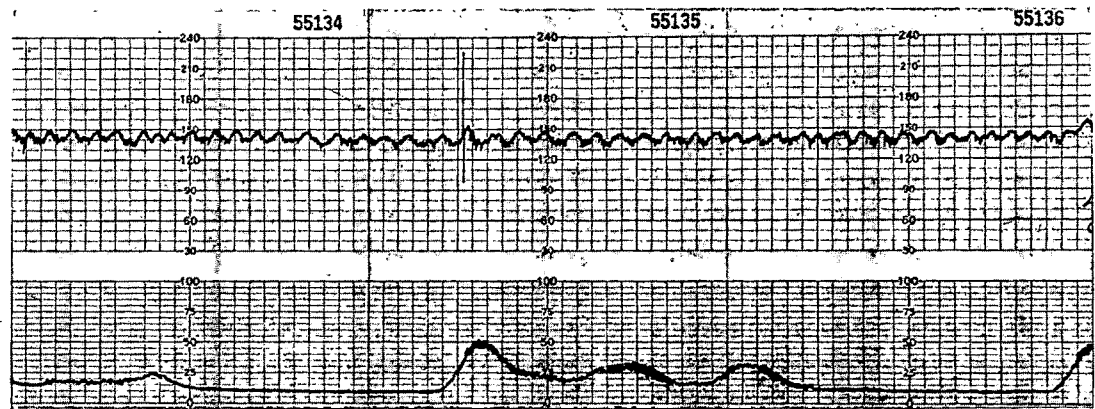


Fig. 1. Sinusoidal fetal heart rate pattern.

Methods and material

All monitored tracings from the first 6-month period of 1977, at Wayne County General Hospital, were reviewed. A total of 551, including 191 external tracings, 283 external plus internal tracings or purely internally monitored tracings, and 77 contraction stress tests (CSTs) composed this study group. The total number of deliveries for this period was approximately 700. The sinusoidal pattern was defined as a rhythmic sine wave pattern, with a frequency of 2 to 5 cpm and an amplitude of 5 to 15 bpm, lasting for at least 2 minutes (Fig. 1). In addition, the charts of all 22 women presenting at Women's Hospital, University of Michigan Medical Center, in 1977, with a pregnancy complicated by rhesus isoimmunization were reviewed and eight tracings of either the antepartum period or the labor period were available for study from these fetuses.

All tracings were assessed for the presence of fetal heart rate (FHR) accelerations, decelerations, and the sinusoidal pattern. Two authors (T. J. and A. A. C.) agreed on the interpretation of all tracings finally classified as sinusoidal. Clinical material was obtained from information documented on the tracings and from maternal and neonatal charts.

Results

The occurrence of the various fetal heart rate patterns in the 551 tracings reviewed at Wayne County General Hospital is summarized in Table I. Although the sinusoidal fetal heart rate pattern could be seen with both external and internal techniques, a clear sinusoidal pattern was often difficult to identify with certainty on the external mode, because of its prevalent "noise." A total of 30 sinusoidal patterns were observed. In 17 cases, the pattern was found to recur on two or occasionally more episodes. Table II shows the duration of the pattern (duration of the longest pattern

Table I. Observed fetal heart rate patterns (N = 551)

| Pattern               | No. | %  |
|-----------------------|-----|----|
| Acceleration          | 336 | 61 |
| Variable deceleration | 202 | 37 |
| Early deceleration    | 48  | 9  |
| Late deceleration     | 18  | 3  |
| Sinusoidal pattern    | 30  | 5  |

Table II. Duration of sinusoidal fetal heart rate pattern (N = 30)

| Duration (min) | No. | %   |
|----------------|-----|-----|
| 2-5            | 8   | 1.5 |
| 5-10           | 6   | 1.0 |
| 10-15          | 6   | 1.0 |
| 15-30          | 7   | 1.3 |
| 30-60          | 2   | 0.3 |
| >60            | 1   | 0.2 |

was used when it was recurrent). In no case did the appearance of the sinusoidal pattern prompt extraordinary obstetric measures. Twenty-nine of the total occurred in tracings from laboring women and the outcome in these cases was uniformly excellent. These 29 cases included three postdate pregnancies, one case of pre-eclampsia, one patient with Class A diabetes, and one heroin addict. All 5-minute Apgar scores were 7 or greater. Only four 1-minute Apgar scores were less than 7, and all four were 6. Three of the latter were associated with midforceps rotation and delivery and one was associated with cesarean section. All neonates had a normal hospital course and were discharged within 4 days, except one infant who remained for surgical repair of gastroschisis. Four cases had scalp pH sampling during labor and no values obtained were less than 7.29.

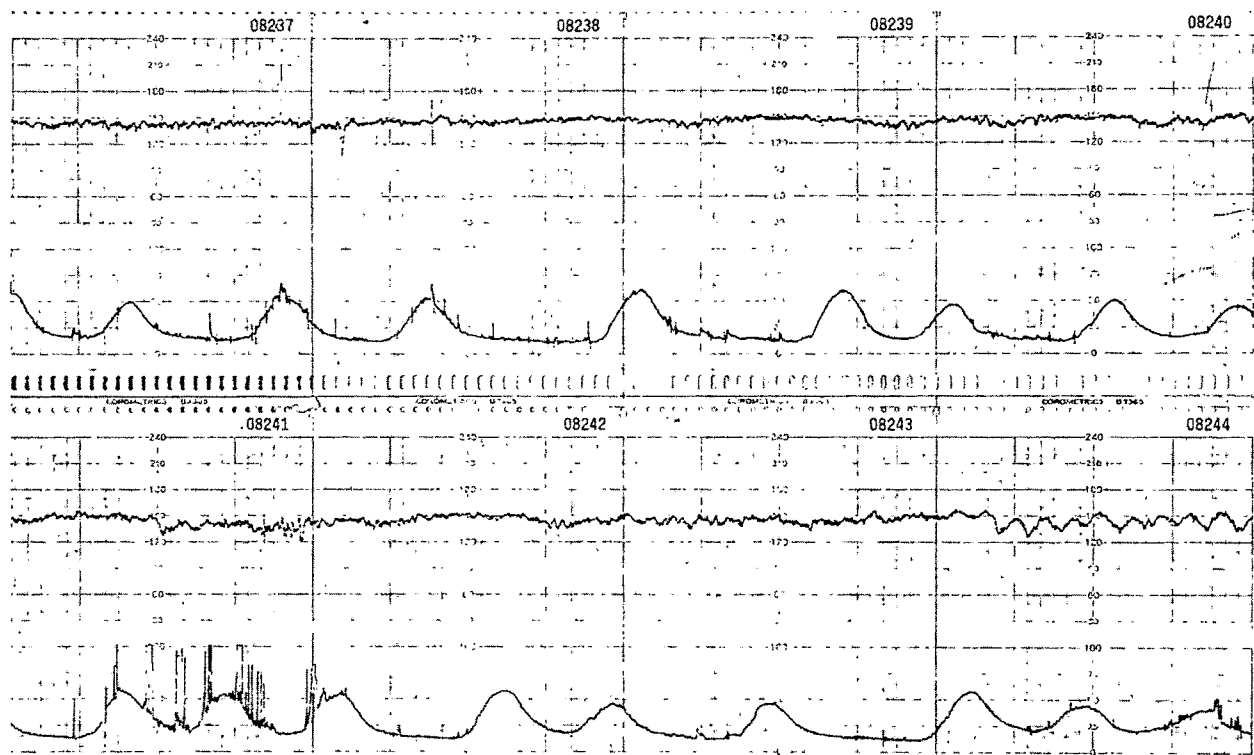


Fig. 2A. Case of sinusoidal fetal heart rate pattern associated with late decelerations and baseline tachycardia with good outcome. See text for details. Figs. 2A, 2B, and 2C are in sequence.

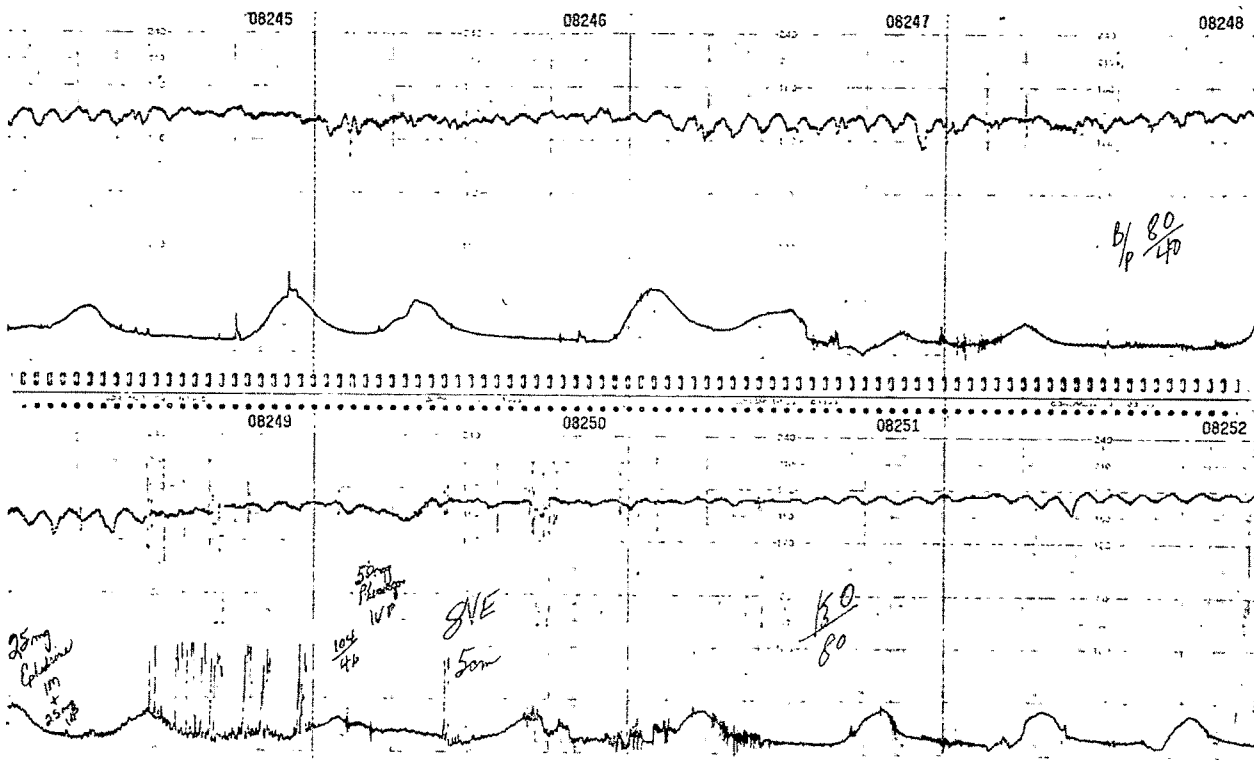
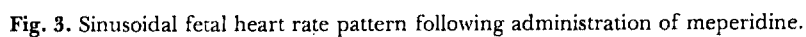
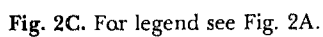


Fig. 2B. For legend see Fig. 2A.





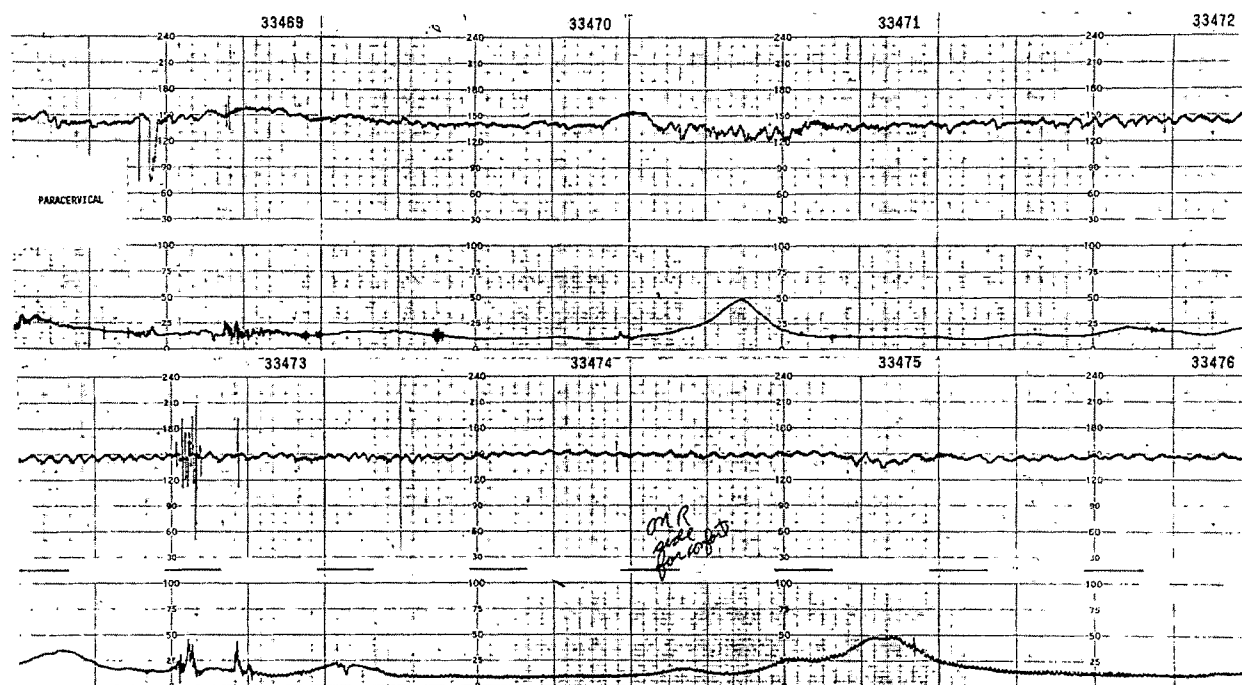


Fig. 4. Sinusoidal fetal heart rate pattern following paracervical anesthesia.

Of interest is that, even when the sinusoidal pattern was associated with known ominous patterns such as late decelerations and marked decreased beat-to-beat variability, the outcome was good, as exemplified by the case in Figs. 2A to 2C. Just prior to the tracing in panel 08237, the patient received an epidural anesthetic. In panels 08239 and 08240, subtle late decelerations were noted. At the end of the next panel (08244), a clear sinusoidal pattern was apparent, and this was associated, as time passed, with a rise in baseline fetal heart rate, a decrease in variability, and the passage of fresh meconium. Biochemical evaluation of the fetus was not done. Panel 14419, recorded some time later, showed a sinusoidal pattern and variable decelerations shortly prior to the delivery of a 35-week, 2,240 gram infant, with Apgar scores of 8 and 9, who had a normal course, except for a successfully repaired gastroschisis.

The single sinusoidal pattern in the CST group was observed in a case complicated by placenta previa and intrauterine growth retardation. Premature labor occurred 2 days later and a 1,190 gram infant was delivered by total breech extraction through Duhrssen's incisions. The Apgar scores were 1 and 1 and the neonate died of documented intraventricular and intracerebral hemorrhage. A tracing shortly prior to delivery was normal, with good accelerative fetal heart rate activity.

Of the eight tracings available for study from rhesus-sensitized pregnancies, one showed the sinusoidal pattern. This tracing was from early labor, at 36

weeks, of a moderately sensitized ( $\Delta$  optical density at 450 nm in Liley's midzone) fetus, delivered shortly thereafter by cesarean section for breech presentation. The Apgar scores were 7 and 8, the central hematocrit was normal, and the neonate did well following exchange transfusions, which were required for hyperbilirubinemia.

In 12 of our cases, the onset of the sinusoidal pattern appeared to be temporally related to administration of analgesics or anesthetics to the mother, usually following within 20 to 30 minutes. In three cases, the drug administered was meperidine (Fig. 3); in five, it was Mepergan (meperidine and promethazine); in two cases, it was morphine sulfate; and in two cases, the pattern was observed following paracervical block with 1% lidocaine (Fig. 4). Although alphaprodine was not used in this study population, experience with this drug on The Johns Hopkins Hospital obstetric service confirms its strong association with the sinusoidal fetal heart rate pattern.<sup>10</sup> In the other 18 cases, the pattern was apparently spontaneous and unassociated with any recognizable obstetric event.

The 30 sinusoidal patterns from Wayne County General Hospital were also analyzed for their association with other fetal heart rate patterns. In 28 tracings (93%) there were associated fetal heart rate accelerations; in 18 (50%), there were variable decelerations; in five (15%), there were early decelerations; and in three (10%), there were late decelerations.



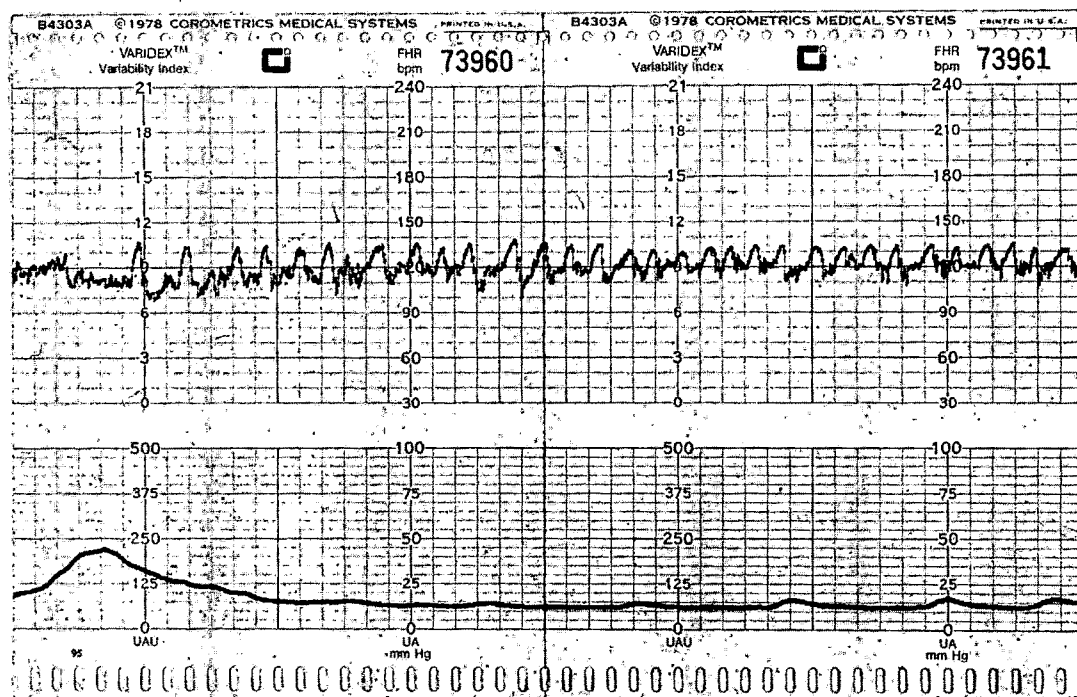


Fig. 5. Sinusoidal fetal heart rate pattern following alphaprodine shows typical jagged configuration.

#### Comment

Any assessment of the occurrence and clinical significance of the sinusoidal fetal heart rate pattern is critically dependent upon several factors. First and foremost is the definition. A waveform is described, in part, by amplitude and frequency measurements. Generally, reports use 2 to 5 cpm and 5 to 15 bpm,<sup>1, 2, 5</sup> but others expand the criteria, especially to include patterns of larger amplitude,<sup>3, 15-17</sup> or fail to specify any criteria.<sup>14</sup> Both decreased oscillation frequency<sup>23, 24</sup> and increased amplitude<sup>3, 14, 15, 17</sup> seem to be consistently associated with poor outcome.

Short-term variability is another critical factor mentioned by only a few.<sup>5</sup> From the examples shown in this report, it is clear that differences in short-term variability may be present within sinusoidal oscillations, and this observation may have the clinical importance it has been shown to have in other clinical circumstances.<sup>25</sup> Manseau and associates<sup>2</sup> report several cases of sinusoidal patterns alternating with a "flat" (absent short-term variability) baseline heart rate.

The baseline rate around which the oscillations occur may also be important. Although this is generally in the 120 to 150 bpm range, reports<sup>7, 24</sup> suggest that when this baseline is significantly lower the prognosis is worse. To make the definition more difficult, there is a certain subjective observer bias used in labeling patterns as sinusoidal. Some sinusoidal patterns are

smoother, more symmetric than others (Fig. 2), and we have observed that the sinusoidal pattern associated with alphaprodine is usually recognizable by its jagged, sawtooth waveform (Fig. 5).

Another critical factor in discussion of waveforms is the duration of the pattern. Even though their outcome is reportedly more favorable,<sup>3</sup> it is hard to imagine why short sinusoidal patterns should be mechanistically different than those of longer duration. We chose to study all patterns greater than 2 minutes and failed to find any association between duration and outcome. This may well reflect the fact that many of our patterns of moderate duration may have been drug induced and, therefore, not directly reflective of an abnormal fetal mechanism. Others have suggested that prolongation of the sinus waveform is ominous,<sup>18</sup> and Katz and associates<sup>16</sup> have shown a fall in tissue pH following appearance of the sinusoidal pattern. Given the problems inherent in the reporting of the sinusoidal fetal heart rate, we propose that future works concentrate more on descriptive morphology (amplitude, frequency, waveform, baseline, duration) when regularly oscillating fetal heart rate patterns are discussed.

Another problem in assimilating previous reports of the sinusoidal pattern is the variety of underlying pathologic conditions described in the cases. There is a clear recurrent association between the sinusoidal pattern and erythroblastosis and other types of fetal

anemia.<sup>1-3, 17</sup> We have also seen a sinusoidal pattern associated with acute fetal hemorrhage and shock. It must be realized, though, that in our infant with erythroblastosis fetalis and others,<sup>8, 10</sup> the pattern did not necessarily augur a bleak outcome. Many reports portray complex cases with multiple possible confounding obstetric risk factors<sup>11, 18-22</sup> that make exact significance of the pattern less clear. Clinical recommendations should be based on cumulative experience rather than individual reports that would tend to emphasize poor outcomes. Ayromlooi and colleagues<sup>14</sup> showed a statistical difference in 1-minute Apgar scores (7.148 versus 8.884) and lower scalp pH (7.288) in those infants showing the sinusoidal patterns when compared to control infants, but the clinical significance of these differences between normal values is unclear. The series of Young and associates<sup>15</sup> showed an incidence of 31% preacidosis or acidosis in fetuses displaying this pattern (usually high amplitude), and others have consistently shown apparently normal outcomes to occur when the pattern is observed,<sup>2, 3, 12, 13, 17</sup> confirming our finding that the pattern is not uniformly ominous.

The pathophysiology of the pattern remains unclear. It is interesting that in this study the sinusoidal pattern was significantly ( $p < 0.001$  by  $\chi^2$  analysis) associated with fetal heart rate accelerations, which are known to reflect good fetal central nervous system reactivity.<sup>26, 27</sup> However, its frequent appearance in conjunction with

narcotic or paracervical anesthetic administration is more consistent with central nervous system deregulation as the cause, as hypothesized in similar occurrences of decreased beat-to-beat variability and bradycardia.<sup>28-30</sup> It would be surprising if multiple factors were not involved. It may be that different oscillatory patterns (amplitude, waveform) are dependent on varied mechanisms.

Thirty-one cases of the sinusoidal fetal heart rate pattern are reported, representing a frequency of 5%. The sinusoidal patterns are often observed following administration of anesthetics, especially narcotic analgesics. Any series will depend on definition and recognition bias for its incidence rate. Further work must concentrate on mechanisms controlling oscillations of the fetal heart rate. Until these are elucidated, and despite the intervening experience, we support the moderate approach espoused by Manseau and associates<sup>2</sup> in their original report. The obstetrician should view the presence of a sinusoidal pattern in the clinical setting of each fetus. Its appearance demands careful assessment of other clinical, electrocardiographic, or biochemical evidence of fetal compromise in order that an appropriate obstetric decision be made.

The patience and exactitude of Ms. P. K. Murphy in the preparation of this manuscript are appreciated.

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# Corticosteroid suppression of estrogen-induced uterine blood flow in nonpregnant sheep

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Estrogenic stimulation of uterine hyperemia and water imbibition are well-described endocrine events. Uterine blood flow responses to estrogen appear to have a similar mechanism of action. Although corticosteroids inhibit the uterine hyperemic and water imbibition effects, their influence on estrogen-induced uterine blood flow has not been clarified. Accordingly, the effects of hydrocortisone on estrogen-induced uterine blood flow were studied in seven nonpregnant castrate ewes equipped with electromagnetic flow probes around the main uterine arteries and with polyvinyl catheters inserted into postbifurcational branches of the uterine arteries. Inferior vena cava infusions of hydrocortisone, 0.50 to 9.25 mg/kg of body weight, administered 1 hour before estrogenic stimulation suppressed uterine blood flow increases by 10% to 35%. Direct uterine intra-arterial infusions of hydrocortisone also inhibited estrogen-induced uterine blood flow at calculated concentrations as low as 5.65  $\mu$ M in uterine arterial blood. These findings support the conclusion that estrogen-induced uterine blood flow and water imbibition are correlative events and further serve to elucidate the mechanisms by which steroid hormones interact to modify uterine blood flow. (AM. J. OBSTET. GYNECOL. 139:454, 1981.)

EARLY UTERINE RESPONSES to estrogenic stimulation consist of vasodilatation, first described by Markee,<sup>1</sup> and increased cellular water imbibition.<sup>2</sup> Subsequent studies using a rat model demonstrated that these two physiologic responses are correlative events.<sup>3</sup> Their similarities were further confirmed by experiments in nonpregnant sheep showing that increases in uterine blood flow have a close time relationship to uterine water imbibition, as well as identical sensitivity to various estrogens.<sup>5</sup> In an attempt to explore the interaction of various steroid hormones, Szego and Roberts<sup>4, 6</sup> observed that administration of glucocorticoids significantly diminishes estrogen-induced uterine water imbibition and hyperemia. However, the effects of glucocorticoids on estrogen-induced uterine

blood flow have not been reported. The purpose of this study was to investigate and quantitate the effect of systemic and uterine arterial administration of glucocorticoids on estrogen-induced uterine blood flow in nonpregnant sheep.

## Material and methods

Seven nonpregnant ewes were used during the study. The anesthetic and surgical procedures have been described in detail.<sup>5, 7, 8</sup> Following sedation with intravenous sodium pentobarbital (5 mg/kg of body weight), spinal anesthesia was induced with the use of Pontocaine Hydrochloride (6 mg). A midline incision was made, and a pelvic laparotomy was performed. The uterine arteries and their lateral branches were exposed by incision of the anterior leaves of the broad ligament. A polyvinyl catheter (inside diameter, 0.58 mm; outside diameter, 0.96 mm) was then inserted into the most lateral postbifurcational branch on each side and advanced retrograde to within 0.5 cm of the bifurcation. Additional catheters were placed into the femoral artery and vein. Electromagnetic flow probes measuring 3 mm were placed around the main uterine arteries proximal to the bifurcation. Following oophorectomy, all catheters and flow probe leads were exteriorized through a fascial incision, subcutaneously

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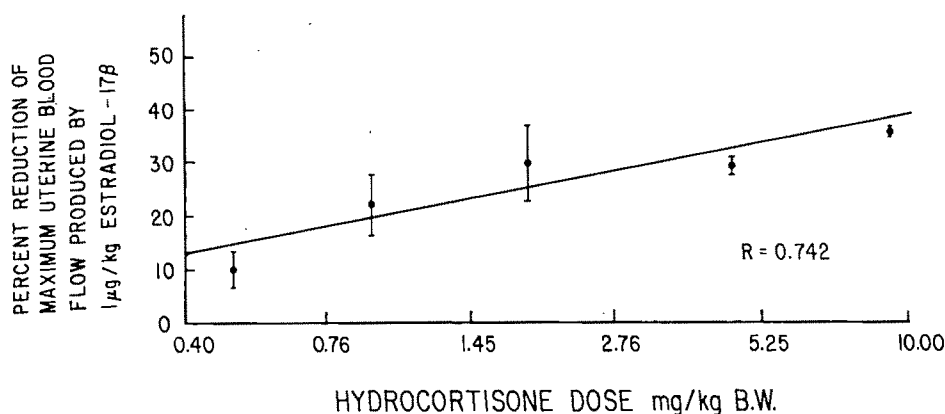


**Fig. 1.** Linear regression of the percentage of reduction of maximum uterine blood flow produced by 1 µg of 17β-estradiol per kilogram of body weight versus a log<sub>10</sub> hydrocortisone dose (milligrams per kilogram of body weight) determined by the method of least squares. The percentage of reduction of the maximum flow =  $20.247 + 18.063 \log_{10}$  hydrocortisone dose (milligrams per kilogram of body weight). Each point represents the mean ± SEM of at least three observations.

tunneled to the skin and stored in a canvas pouch.

After surgery, each sheep was placed in a rectangular stall and remained in the laboratory for the remainder of the study. Micron R. C. 1000 electromagnetic flowmeters\* were used to measure uterine arterial blood flow. These signals were integrated electronically and displayed on a pen recorder running at 1 mm/minute. Systemic arterial pressure was measured by means of a Statham p23-Db transducer and displayed on the same recorder.

Previous studies had demonstrated that reproducible maximal uterine arterial vasodilatation could be achieved either by systemic administration of 1 µg/kg of 17β-estradiol or by direct uterine intra-arterial injection of 1 µg of 17β-estradiol.<sup>5, 7</sup> For this reason, the peak blood flow responses to these doses were used as reference points, and the response observed during each hydrocortisone infusion was expressed as the percentage of reduction of maximum estrogen-induced uterine blood flow recorded from that preparation on the day prior to study. Hydrocortisone infusions were not begun until maximal uterine vasodilatation was consistently recorded in each preparation.

The 17β-estradiol† was of reagent-grade quality and was dissolved in absolute alcohol and diluted in 0.9% sterile saline to a concentration of 1 µg/ml in the working solution. The ethanol concentration used has been demonstrated to have no effect on uterine blood flow.<sup>7</sup> Hydrocortisone sodium succinate‡ dissolved in benzyl

alcohol and sterile water was diluted in 0.9% sterile saline for continuous infusion.

In the first series of 18 experiments, infusion of hydrocortisone was begun with a constant-infusion pump into the inferior vena cava following measurement of baseline uterine blood flow. After 60 minutes, 17β-estradiol, 1 µg/kg of body weight, was given intravenously over 1 minute and the hydrocortisone infusion was continued for an additional 60 minutes. Peak uterine blood flow on each side was then recorded and expressed as a percentage of reduction from the previous day's peak estrogen response. Hydrocortisone infusions ranged from 0.50 to 9.25 mg/kg of body weight. In the second set of 27 experiments, the protocol was the same except that hydrocortisone was infused locally into a uterine artery. Arterial concentrations of hydrocortisone were calculated on the basis of control blood flows and ranged from 5.65 µM to 1.13 mM. The contralateral horn was infused with 0.9% saline as a control. Uterine arterial responses were recorded and expressed as previously described.

## Results

In each experiment, the intravenous infusion of hydrocortisone resulted in suppression of peak estrogen-induced uterine blood flow. The degree of suppression was dose related ( $r = 0.742$ ) and the dose-response curve is depicted in Fig. 1. Over the range of 0.50 to 9.25 mg/kg of hydrocortisone infused, uterine blood flow increases were suppressed by 10% to 35%. The qualitative characteristics of the estrogen-induced response in this preparation, as described previously,<sup>6, 7</sup> were not altered by hydrocortisone.

\*Micron Instrument Co., Los Angeles, California.

†Sigma Chemical Co., St. Louis, Missouri.

‡The Upjohn Co., Kalamazoo, Michigan.

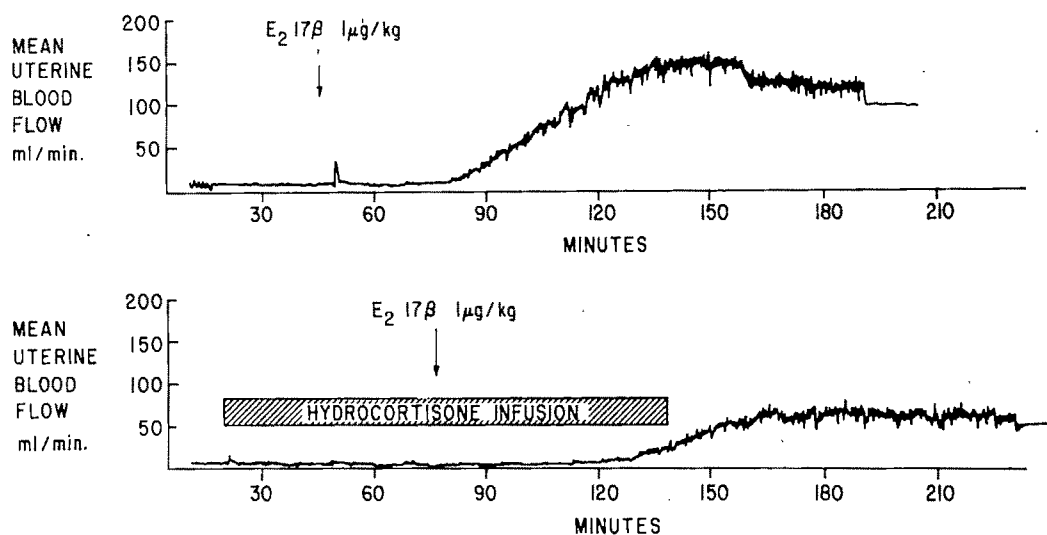


Fig. 2. Comparative uterine arterial flow responses with and without uterine arterial hydrocortisone infusion (total dose 120 mg, 1 mg/min).

Direct uterine intra-arterial infusions of hydrocortisone also inhibited estrogen-induced uterine blood flow responses at all concentrations studied, although this did not appear to be dose related. Mean suppression was  $44.4\% \pm 2.3\%$ . At the lowest calculated concentration ( $5.65 \mu\text{M}$ ) suppression greater than 30% was consistently noted. An example of the typical estrogen-induced increase in uterine blood flow and its suppression by uterine arterial hydrocortisone infusion is seen in Fig. 2.

No changes in mean arterial pressure were detected either during or after hydrocortisone infusions, and baseline uterine blood flow was also unchanged by the infusions.

#### Comment

Dilatation of the uterine vasculature in response to estrogenic stimulation has been well described by several investigators.<sup>1, 4, 9</sup> An increase in uterine wet weight secondary to the intracellular accumulation of water also has been shown to occur shortly following estrogen administration.<sup>2</sup> Holden's<sup>3</sup> observation of the concurrent nature of these two physiologic responses formed the basis of the concept that they are physiologically similar events. Further evidence supporting this idea came with the finding that the relative potencies of various estrogens for stimulating increased blood flow and water imbibition are similar in contrast to their relative abilities in promoting other uterine manifestations of estrogen administration.<sup>5</sup> It is of additional significance that exposure of the nonpregnant sheep

uterus to progesterone causes a significant reduction in estrogen-induced uterine blood flow, in a manner quantitatively similar to progesterone suppression of estrogen-induced uterine water imbibition.<sup>10, 11</sup>

Antagonism to estrogenic stimulation by the pituitary-adrenal system was first described by Szego and Roberts,<sup>4, 6, 12</sup> measuring water imbibition (early wet weight) in the rat uterus. They initially found that adrenalectomy enhanced uterine water imbibition and conversely that adrenocorticotrophic hormone administration caused a marked suppression which could be abolished by adrenalectomy.<sup>12</sup> Similar degrees of inhibition were found with the use of systemic injections of various corticosteroids; of those studied, hydrocortisone appeared to be the most effective in blunting this response.<sup>4, 6</sup> The present findings confirm in a quantitative manner that corticosteroids significantly suppress estrogen-induced increases in uterine blood flow and, in view of the earlier observations, support the conclusion that these responses are similar physiologic events.

The mechanisms by which corticosteroids modify uterine water imbibition and blood flow are unclear at this time. Hechter and associates<sup>13</sup> noted an increase in uterine capillary permeability in response to estrogen. In two separate studies<sup>14, 15</sup> cortisone administration to rats decreased cellular and vascular permeability. Subsequent experiments by Spaziani and Szego<sup>16</sup> demonstrated the ability of hydrocortisone to block intracellular movement of both water and sodium in response to estrogen. Although corticosteroid-mediated reduction



in capillary permeability may explain the antagonism to water imbibition, it does not provide an explanation for the reduction in uterine blood flow.

It is highly unlikely that the observed suppression can be explained by competitive binding of glucocorticoids with estrogen on cellular sites or a glucocorticoid-induced reduction in their number. This latter mechanism may well explain the ability of progesterone to diminish estrogen-induced increases in both uterine blood flow and water imbibition, since progesterone has been demonstrated to deplete the quantity of cytoplasmic estrogen receptor.<sup>10, 11</sup> However, these hypotheses are not tenable vis à vis corticosteroid suppression since it has been shown that the sow estrogen receptor in vitro is highly stereospecific and that corticosteroids display no binding to this receptor.<sup>17</sup> The inability of corticosteroids to compete with estrogen for available receptors was also demonstrated by Clark et al.<sup>18</sup> who showed that hydrocortisone treatment has no effect on the quantity of either cytoplasmic or nuclear estrogen receptors in adult castrated rats. Although Terenius<sup>19</sup> has observed that pretreatment of the mouse uterus with hydrocortisone does not inhibit the uptake of labeled 17 $\beta$ -estradiol, Campbell<sup>20</sup> was able

to demonstrate both an inhibition of estrogen uptake and a reduction in the quantity of nuclear-bound receptor-estrogen complex in rats pretreated with dexamethasone. He postulated an interaction of corticosteroid with the plasma membrane to restrict entry of estrogen into target tissue.

Recent work from our laboratory<sup>8, 21</sup> suggests that prostaglandins may be involved in mechanisms which regulate estrogen-induced blood flow increases in the nonpregnant uterus. There is abundant evidence in both in vitro and in vivo systems that corticosteroids inhibit the cellular elaboration of prostaglandins.<sup>22-26</sup> While there is general agreement that glucocorticoids do not decrease prostaglandin synthetase activity, there is divergence of opinion concerning the exact location of the antagonism.<sup>22, 24</sup> Various evidence suggests that glucocorticoids may act by reducing cellular release of prostaglandins<sup>26</sup> or diminishing the availability of arachidonic acid to the microsomal cyclooxygenase system.<sup>23, 25</sup> If vasodilatory prostaglandins are ultimately determined to mediate estrogen-induced uterine blood flow, it is possible that the glucocorticoid suppression may be explained by this mechanism.

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# Ontogenesis of beta-adrenergic receptors in the ovine placenta

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Radioligands with high specific activity and high affinity have recently become available to study the  $\beta$ -adrenergic receptor (BAR). BAR may be important in the placenta for mediating a variety of metabolic and hemodynamic effects of catecholamines including placental hormone synthesis and secretion, placental glycogenolysis, and placental blood flow. Little is known regarding the development of the BAR. We have used the tritiated radioligand dihydroalprenolol to study the ontogenesis of BAR in the fetal and maternal portions of the ovine placenta. The receptor number decreased from 415 fmol  $\cdot$  mg<sup>-1</sup> of protein at 120 days' gestation to 226 fmol  $\cdot$  mg<sup>-1</sup> at 145 days; this trend was highly significant ( $r = -0.822$ ,  $p < 0.01$ ). Similar changes were noted in the dissociation constant. Downward regulation as a result of fetal-neurosympathetic maturation is proposed as an explanation for this observation. (AM. J. OBSTET. GYNECOL. 139:459, 1981.)

THE TRADITIONAL VIEW of catecholamine physiology holds that neurally or humorally transmitted biogenic amines interact with target cells by binding to surface "receptors" located within the cell membrane. This highly stereoselective, high-affinity binding induces the subsequent physiologic alterations characteristic of the target tissues. On the basis of the responses elicited and the relative potency of different catecholamines, Ahlquist<sup>1</sup> concluded that there must be two types of receptors, which he termed "alpha" and "beta." Subsequently a wealth of pharmacologic data has been developed that extends these observations with the use of various agents to stimulate or block selectively the action of the catecholamine on these putative receptors. With the recent development of synthetic compounds of high specific activity and binding affinity and with stereoselectivity characteristic of their naturally occurring analogues, it has become possible to study adrenergic receptors directly.<sup>2</sup>

Whitsett and colleagues<sup>3</sup> have demonstrated the presence of beta-adrenergic receptors (BAR) in the human placenta. However, because of the heterogeneity of patients available at a variety of gestational ages and their concomitant pathologic conditions, controlled study of the developmental changes in human placental BAR is not possible. Thus, we have studied the development of BAR in the ovine placenta with the use of the tritiated radioligand dihydroalprenolol (DHA).

## Material and methods

At the designated gestational ages, date-bred Rambouillet-Columbia ewes were delivered by cesarean section with the ewe under spinal or epidural anesthesia. Total hysterectomy was then performed. Membrane preparation and binding assay were done with a modification of published methods.<sup>2,3</sup> Placental tissue was removed rapidly and chilled in iced homogenization buffer (250 mM sucrose; 1 mM ethyleneglycol-tetra-acetic acid, and 10 mM tromethamine-hydrochloric acid, pH 7.4). Excess membranes were removed from the cotyledons and the maternal and fetal portions were separated by blunt dissection. Receptors were purified as spherical membrane fragments by homogenization and ultracentrifugation. An electron micrograph of the final preparation used in direct

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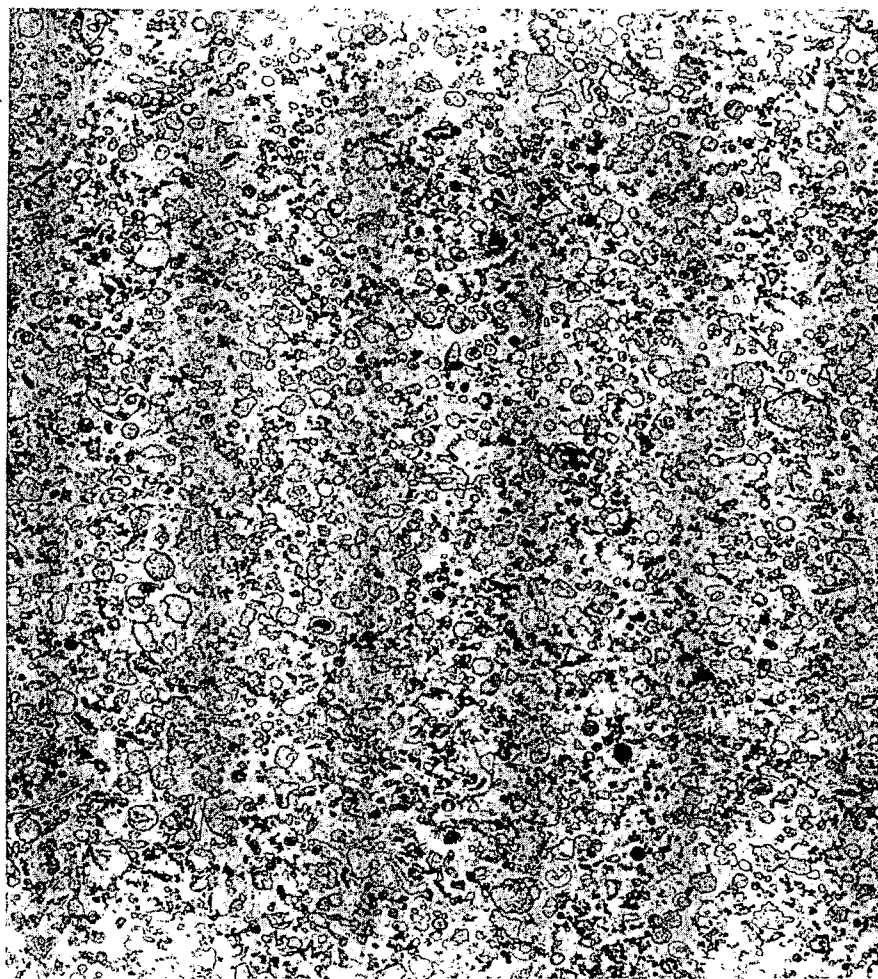


**Fig. 1.** Low-power electron micrograph of membrane preparation demonstrating spherical membrane vesicles and mitochondrial and subcellular debris fragments. ( $\times 4,000$ .)

binding studies is shown in Fig. 1. As can be seen, this preparation consists largely of spherical membrane vesicles with some mitochondrial and subcellular debris fragments ( $\times 4,000$ ).

Direct binding studies were done in 50 mM tromethamine-hydrochloric acid, pH 7.4, and 10 mM magnesium chloride with concentrations of [ $^3\text{H}$ ] DHA (New England Nuclear, specific activity 45 Ci/mmol) ranging from 0.5 to 10 nM. Bound and free material was separated by rapid filtration and washing on Whatman GF/C glass fiber filters and counted in a liquid-scintillation system at 52% efficiency. Nonspecific binding was defined as the amount of DHA bound in the presence of 1  $\mu\text{M}$  DL-propranolol. All assays were performed in duplicate and repeated three to six times. Interassay variability was 10% to 15%. The protein concentration of the membrane preparation was determined by the method of Lowry and associates.<sup>4</sup>

Binding capacity or receptor number, expressed as

femtomoles of DHA bound per milligram of membrane protein, and the dissociation constant,  $K_d$ , were determined by Scatchard<sup>5</sup> analysis of the direct binding data. The dissociation constant also was determined by kinetic analysis of the binding data. Competitive binding curves were determined under similar assay conditions with addition of agonists over the designated ranges (L-epinephrine bitartrate, L-norepinephrine bitartrate, and L-isoproterenol hydrochloride were purchased from Sigma Chemical Co.) (Fig. 2).

### Results

A representative saturation curve for  $^3\text{H}$ -DHA showing total and nonspecific binding is shown in Fig. 3. The Scatchard plot is shown in the insert. Linear regression analysis was used to approximate the binding capacity ( $x$ -intercept) and dissociation constant (negative reciprocal of slope).

Receptor studies were performed in three prepara-



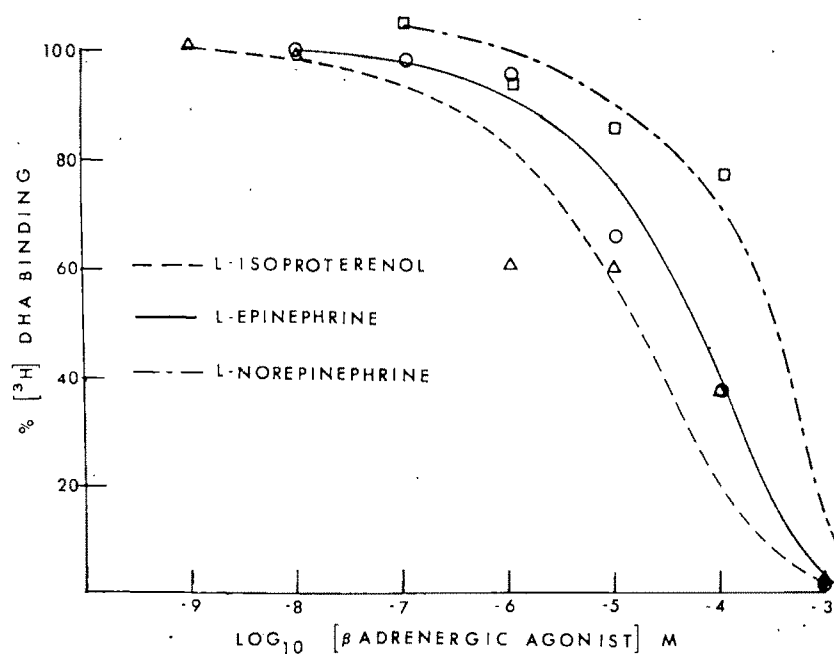


Fig. 2. Competitive binding curves for unlabeled (—) isomers at specified concentrations with [ $^3$ H]-DHA, 4 nM. Incubations were carried out as described in Material and methods.

Table I. Binding capacity versus gestational age

|                                                              | Gestational age (days)     |                          |                          |
|--------------------------------------------------------------|----------------------------|--------------------------|--------------------------|
|                                                              | 120                        | 130                      | 145                      |
| Binding capacity (fmol $\cdot$ mg $\cdot$ protein $^{-1}$ *) |                            |                          |                          |
| Fetal                                                        | 415.3 $\pm$ 35.24†<br>(3‡) | 382.0 $\pm$ 37.00<br>(2) | 226.3 $\pm$ 40.08<br>(4) |
| Maternal                                                     | 184.3 $\pm$ 31.89<br>(3)   | 104.0 $\pm$ 4.0<br>(2)   | 104.0 $\pm$ 38.00<br>(2) |
| Dissociation constant, Kd (nM)§:                             |                            |                          |                          |
| Fetal                                                        | 5.5 $\pm$ 0.23*<br>(3)     | 3.98 $\pm$ 1.12<br>(2)   | 2.21 $\pm$ 0.47<br>(4)   |
| Maternal                                                     | 2.55 $\pm$ 0.37<br>(3)     | 1.49 $\pm$ 0.11<br>(2)   | 1.18 $\pm$ 0.76<br>(2)   |

\*Regression of binding capacity on gestational age for fetal receptors was significant ( $r = -0.822$ ,  $p < 0.01$ ). Maternal studies:  $r = -0.644$ ,  $p < 0.1 > 0.05$ .

†Mean  $\pm$  SEM.

‡Number of preparations studied.

§Regression of dissociation constant on gestational age for fetal studies was significant ( $r = -0.879$ ,  $p < 0.01$ ). Maternal preparation:  $r = -0.714$ ,  $p < 0.10 > 0.5$ .

tions at 120 days' gestation, two at 130 days' gestation, and four at 145 days' gestation. Table I contains the observed binding capacities and dissociation constants at each gestational age. There was a significant decrease in binding capacity as gestation advanced ( $r = 0.822$ ,  $p < 0.01$ ). Similarly, there was a significant decrease in dissociation constant with advancing gestation ( $r = 0.879$ ,  $p < 0.01$ ). Maternal receptor binding showed the same trend of decrease in binding capacity with gestational age, but the decrease was not significant. Receptor numbers were less in maternal tis-

sue, at each gestational age, than in the fetal tissues. Kinetic analysis of direct binding studies showed good correlation with results determined at equilibrium, 2.42 and 2.21 nM, respectively. Agonist competition curves with  $^3$ H-DHA showed the order of potency isoproterenol  $>$  epinephrine  $>$  norepinephrine typical of  $\beta_2$ -receptors.

#### Comment

Developmental changes in cell membrane BAR have been reported in several systems. In the fetal mouse

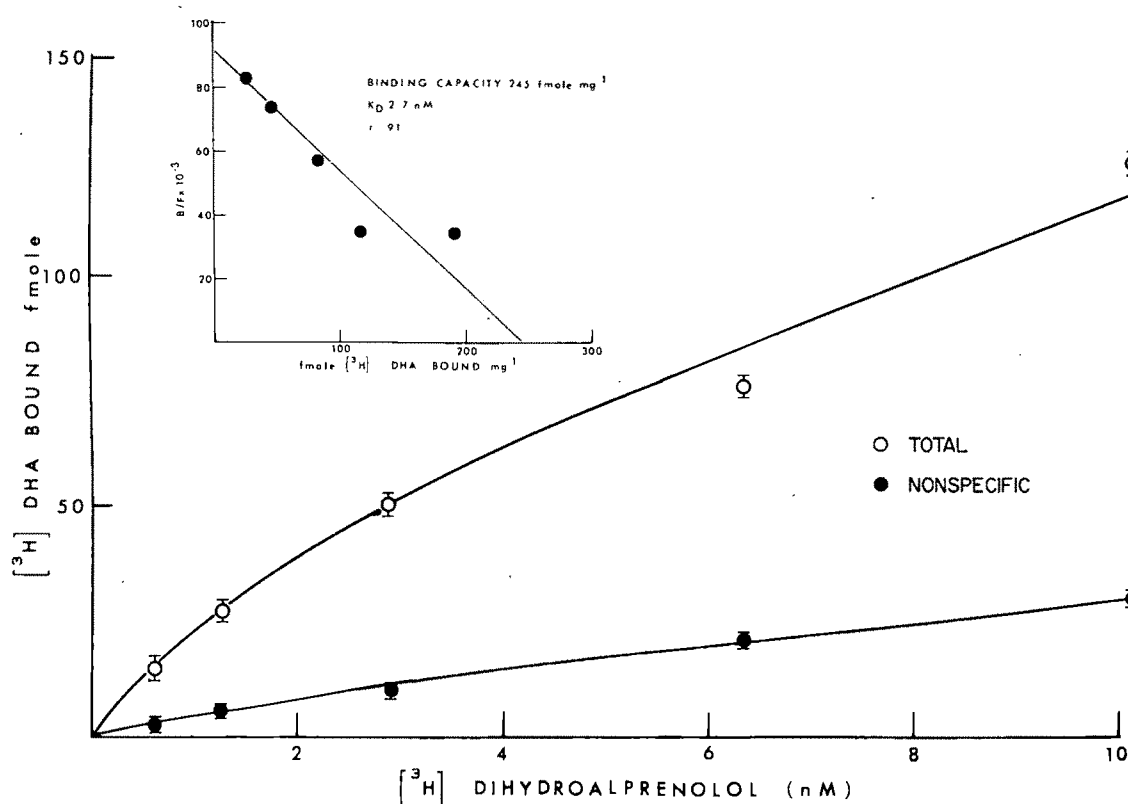


Fig. 3. Saturation binding curve for  $[^3\text{H}]$ -DHA, concentration 0.5 to 10 nM. Nonspecific binding is in presence of  $10^{-6}\text{M}$  propranolol. Incubation was for 20 minutes. Values represent mean  $\pm$  SEM for duplicate determinations repeated three times. Inset: Scatchard plot, in femtomoles per milligram of protein.<sup>-1</sup>

heart<sup>6</sup> BAR increase with advancing gestation and are demonstrable prior to the onset of isoproterenol-mediated changes in heart rate. Likewise, in the fetal rabbit lung<sup>7, 8</sup> there is an increase in BAR with advancing gestation which could be accelerated by glucocorticoid therapy. In the rat cerebral cortex catecholamine-stimulated adenylyl cyclase activity and BAR appear concomitantly with the rapid development of the postsynaptic response to norepinephrine in the cerebral cortex.<sup>9</sup> Thus, it seems that developmental changes in the receptor, the adenylyl cyclase system, or both can explain some of the observed alterations in adrenergically mediated physiologic processes associated with development.

In the present study we observed a decrease in BAR with advancing gestation which we postulate reflects "downward regulation" as the result of maturation of the fetal adrenergic nervous system. This decrease in receptor number or "downward regulation" in response to sustained exposure of the receptor to catecholamines has been observed in a variety of situations<sup>10-12</sup> and is postulated to be a mechanism for the phenomenon of desensitization. There is good evi-

dence that maturation of the fetal adrenergic nervous system is associated with increased catecholamine production rate and responsiveness.<sup>13-15</sup> Our laboratory has recently reported an increase in catecholamine production rate and metabolic clearance with advancing gestation.<sup>16</sup> In a recent study characterizing the human placental BAR,<sup>3</sup> an apparent decrease in binding capacity was observed in those preparations exposed to high levels of  $\beta$ -sympathomimetics during tocolysis. However, there were an insufficient number of preparations or controls for the study to be conclusive.

The implications of a decrease in receptor number are perhaps reflected in the functions of placental BAR might subserve. Adrenergic stimulation of the placenta has been shown to have numerous effects, including increased progesterone synthesis,<sup>17</sup> decreased human placental lactogen secretion,<sup>18</sup> increased glycogenolysis,<sup>19</sup> increased cyclic adenosine monophosphate-mediated estrogen synthesis, human chorionic gonadotropin secretion,<sup>20</sup> and regulation of placental blood flow.

Adenylyl cyclase has been identified in the human placenta<sup>4</sup> and is presumed to interact predominantly



with catecholamines of fetal origin. The fetal or maternal origin of the catecholamine governing each of these effects is unclear at present; however, indirect evidence suggests that small amounts, at best, traverse the placental barrier.<sup>21</sup> We believe the difference in receptor number between the fetal and maternal tissues reflects the fetal origin of these receptors. While the placental tissue separates easily from the myometrium because of projection of the fetal vasculature into the maternal tissue, significant contamination with fetal tissue is likely to have occurred. That the receptor is located on the fetal side of the circulation has been suggested by localization of the adenylate cyclase activity and differential purification of brush border membranes in the human placenta; these procedures enriched binding and cyclic adenosine monophosphate production in the non-brush border membranes opposing the fetal circulation.<sup>4</sup>

The umbilical circulation is distinctive in lacking innervation. Previously it has been assumed that there was likewise no humoral adrenergic regulation of the placental circulation. However, recent evidence both *in vitro*, with vascular strips demonstrating vascular reactivity characteristic of the BAR,<sup>22</sup> and *in vivo*, with

$\beta$ -blockade in the chronically catheterized ovine fetus, suggests that the umbilical placental circulation is under tonic  $\beta$ -adrenergic regulation.<sup>23</sup> This is consistent with the numerous earlier observations that the placental vascular bed is under significant active regulation and that perturbations such as exercise or hypoxia can have significant effects on the degree and balance of placental exchange. During hypoxia the umbilical placental vascular bed can be shown to be under maximal  $\beta$ -stimulation<sup>24</sup> and placental shunting or mismatch in placental circulation is minimized.<sup>25</sup>

The significance of the decreasing placental adrenergic receptor number with advancing gestation and the modulation of each of these processes are unclear at present. It is intriguing to speculate on the role of adrenergically mediated placental progesterone synthesis, decreasing receptor number, and the timing of the onset of labor. A decrease in catecholamine-mediated regulation of placental hemodynamics may underlie the observed increased susceptibility of term neonates to hypoxic, intrapartum insults.<sup>26</sup> Thus, these ontogenic studies may provide a clue to our understanding of perinatal phenomena.

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# Glucocorticoids, hyperinsulinemia, and fetal lung maturation

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Glucocorticoids are reported to accelerate fetal lung development, whereas insulin is alleged to interfere with this effect of glucocorticoids. A paradox exists, however, in that glucocorticoids also induce hyperinsulinemia. The purpose of this study was to explore the interrelationships of betamethasone, hyperinsulinemia, and hyperglycemia to fetal lung maturation. In this rhesus preparation, maternal betamethasone administration produced an alarming increase in maternal and fetal plasma insulin values. A significant increase in total lung volumes also occurred, but lung surfactant properties (as measured by amniotic fluid lecithin/sphingomyelin concentrations, lung alveolar deflation stability, and lung phosphatidylcholine concentrations) remained unchanged. These findings are consistent with the following hypotheses: (1) Betamethasone-induced hyperinsulinemia impairs acceleration of surfactant production but does not negate increases in maximum lung volume; (2) betamethasone-induced increases in maximum lung volume occur through mechanisms other than alveolar surfactant alterations. (AM. J. OBSTET. GYNECOL. 139:465, 1981.)

THERE IS INCREASING EVIDENCE that glucocorticoids play an important role in fetal lung maturation and that insulin may interfere with this action. Glucocorticoids reportedly accelerate lung maturation, as measured by increased lung lecithin concentration, in-

creased alveolar deflation stability, increased lung distention, and morphologic changes.<sup>1</sup> Not only have administered glucocorticoids been reported to produce these changes, but also it has been suggested that endogenous glucocorticoid production at the end of pregnancy may accomplish the same phenomena.<sup>2, 3</sup>

On the other hand, the respiratory distress syndrome is reported to occur with increased frequency in infants of diabetic mothers, even when gestational age, method of delivery, sex, and Apgar score are taken into account.<sup>4</sup> The high insulin levels known to occur in infants of diabetic mothers have been suggested as possibly interfering with lung maturation. Draisey and associates<sup>5</sup> measured amniotic fluid immunoreactive insulin concentrations and lecithin concentrations and found that a reverse correlation existed between these two components of amniotic fluid. They concluded that these results supported the hypothesis that insulin inhibits lecithin synthesis.

Smith and associates,<sup>6</sup> using monolayer cell cultures from 28-day fetal rabbit lungs, found that the addition of insulin had a slight positive effect on choline incorporation into lecithin. In contrast, when cultures were

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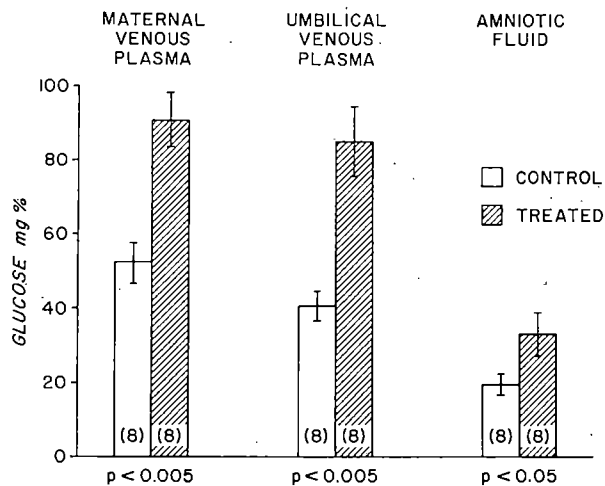


**Fig. 1.** The effect of maternal betamethasone treatment on glucose concentrations in the three compartments: maternal, fetal, and amniotic fluid. Open bars represent the control group; shaded bars, the 3-day treated group. The numbers of observations are indicated in parentheses; the vertical bars represent  $\pm$  the standard error of the mean.

incubated in the presence of cortisol, all concentrations of insulin significantly reduced lecithin synthesis. Smith and associates concluded from their data that hyperinsulinemia, characteristic of the infant of the diabetic mother, might interfere with the physiologic process of glucocorticoid-induced pulmonary maturation and thereby increase the risk of the respiratory distress syndrome for these infants.

Neufeld and co-workers,<sup>7</sup> using fetal rabbit lung slices, observed that insulin reduced the incorporation of glucose and fatty acid residues into saturated phosphatidylcholine. Rhoades and associates<sup>8</sup> reported that lung slices from neonatal rats born to diabetic mothers showed a reduction in incorporation of glucose into neutral lipids and a reduced oxidation to carbon dioxide. Gross and Smith<sup>9</sup> demonstrated that insulin delayed morphologic maturation of the fetal rat lung in vitro, which also suggests an inhibitory effect of insulin on normal lung development.

In additional in vitro studies, Wolfe and associates<sup>10</sup> concluded that insulin indirectly affected surfactant synthesis by reducing substrate through its inhibitory effect on lipolysis. Therefore, there are a number of studies supporting the concept that hyperinsulinemia interferes with the metabolic processes by which undifferentiated lung tissue undertakes surfactant production and becomes functional.

However, there is an apparent contradiction. The administration of glucocorticoids is well known to be associated with hyperinsulinemia.<sup>11</sup> These observers may not have given consideration to the fact that in the

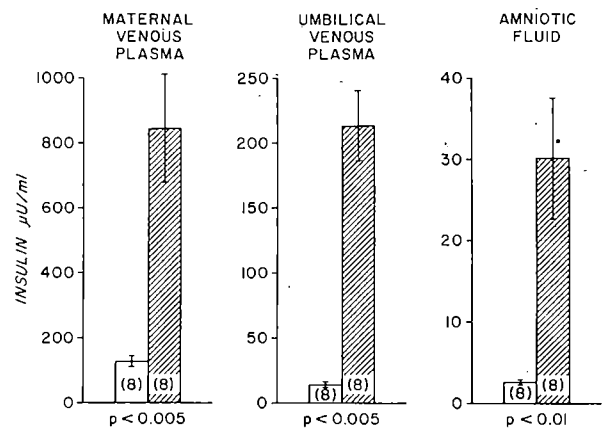


**Fig. 2.** The effect of betamethasone treatment on insulin concentrations in the maternal, fetal, and amniotic fluid compartments. Open bars represent the control group; shaded bars, the treated animals. The numbers of observations are indicated in parentheses; the vertical bars represent  $\pm$  the standard error of the mean.

intact animal the administration of glucocorticoids is associated with marked fetal hyperinsulinemia. If glucocorticoids do induce surfactant production but also promote hyperinsulinemia, what is the mechanism by which accelerated lung development occurs?

The purpose of this in vivo study was to determine what relationship glucocorticoid-induced hyperinsulinemia might have to fetal lung maturational characteristics. In particular, the study was designed to explore the effects on the amniotic fluid lecithin/sphingomyelin (L/S) ratio, maximum lung volume, alveolar deflation stability, and lung phosphatidylcholine concentrations.

#### Material and methods

Sixteen pregnant rhesus monkeys (eight pairs of *Macaca mulatta*), matched for duration of gestation, were used for these studies. Details regarding the determination of the duration of pregnancy and the care of the pregnant animal have been reported previously.<sup>12</sup> The pregnant animals were separated into two groups: Group 1 (control mothers) received isotonic saline (Med Tech, Inc., Elwood, Texas) intramuscularly (0.3 ml) daily for 3 days prior to preterm delivery, and Group 2 (treated mothers) received 1 mg of betamethasone phosphate and 1 mg of betamethasone acetate (Celestone Soluspan, Schering Corp., Kenilworth, New Jersey) intramuscularly (0.3 ml) for the same duration of time. This daily dosage, which is one sixth that used in clinical obstetrics, was chosen on the basis that the rhesus fetal weight is about one sixth that of the human fetus. Gestational age at delivery was chosen to vary between 67% and 85% of term preg-

nancy, comparable in time to 29 to 34 weeks of human pregnancy. Group 1 and Group 2 contained one animal each, delivered at 110, 115, 120, 125, 130, 133, 135, and 140 days' gestation. Delivery was accomplished by cesarean section in all cases, according to previously described techniques.<sup>12</sup> Amniotic fluid samples and maternal venous and umbilical venous blood samples were obtained at the time of delivery. The fetal trachea was clamped upon delivery to prevent spontaneous fetal lung inflation. Death occurred shortly thereafter. All data were collected without prior knowledge as to the gestational age or treatment regimen of the animal. Fetal body weight and sex were determined.

The amniotic fluid samples were processed and the L/S ratio was determined as described previously.<sup>12</sup>

**Pressure volume studies.** Following fetal death by asphyxiation, the trachea was unclamped and cannulated. Pressure volume studies were then performed with the thorax intact, by previously described methods.<sup>12</sup> The values for pressure volume analyses were taken from the second inflation and deflation limbs. Maximal lung volume was determined at a transpulmonary pressure (TPP) of 40 cm of water and expressed as milliliters of air per kilogram of body weight. Proportional volume changes during inflation and deflation at predetermined airway pressures were expressed as the percentage of maximum lung volume achieved at 40 cm of water. The latter volume was designated as 100%.

#### Biochemical studies.

**Insulin and glucose assays.** Plasma and amniotic fluid concentrations of glucose were measured by the glucose oxidase method. Plasma and amniotic fluid insulin levels were determined by the two-antibody radioimmunoassay technique.<sup>13</sup> The first antibody used was guinea pig antiporcine.

The interassay coefficient of variation was 9% to 10% and the intra-assay coefficient of variation was 6% to 7%.

The deoxyribonucleic acid (DNA) and phospholipid analyses on lung tissue were performed as previously described.<sup>12</sup> Statistical significance for all measurements was assessed by the paired t test.

#### Results

After all studies had been completed, the code was obtained to determine the gestational ages and treatment program and the animals were assigned to either the treatment or the control group. Statistical analyses were then done to determine the significance of differences between groups and to test correlations between various measurements among groups.

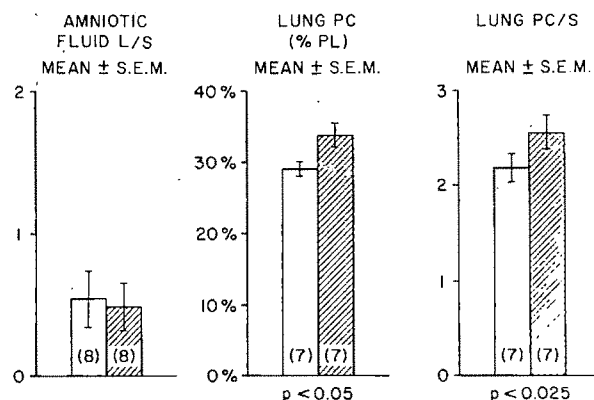


**Fig. 3.** Biochemical data on phospholipid content in the amniotic fluid compartment and in the whole-lung tissue. Open bars represent the control group; shaded bars, the treated group. The numbers of observations are indicated in parentheses; the vertical bars represent  $\pm$  the standard error of the mean. PC = Phosphatidylcholine; PL = phospholipid; and S = sphingomyelin.

In the treated animals, the maternal, fetal, and amniotic fluid glucose concentrations were about twice those noted in the control population. The differences were as follows: maternal venous plasma glucose =  $90.9 \pm 20.7$  mg/100 ml (mean  $\pm$  SD) and  $52.3 \pm 15.5$  mg/100 ml ( $p < 0.005$ ); umbilical venous plasma glucose =  $85 \pm 26.4$  mg/100 ml versus  $40.5 \pm 11.0$  mg/100 ml ( $p < 0.005$ ); amniotic fluid glucose =  $33 \pm 16.4$  mg/100 ml versus  $19.4 \pm 7.7$  mg/100 ml ( $p < 0.05$ ) (Fig. 1).

Concomitant with this rise in glucose concentrations among the treated animals was a significant increase in the insulin concentrations (Fig. 2). Animals receiving betamethasone had a mean maternal venous insulin concentration of  $845.8 \pm 470.3$   $\mu$ U/ml (mean  $\pm$  SD) as compared to the control value of  $129.7 \pm 49.1$   $\mu$ U/ml ( $p < 0.005$ ), an increase of greater than sixfold. Treated fetuses had umbilical venous plasma insulin levels of  $213.6 \pm 77.9$   $\mu$ U/ml compared to a control level of  $13.8 \pm 5.4$   $\mu$ U/ml ( $p < 0.005$ ), an increase of fifteenfold. The amniotic fluid insulin concentration in the treated animals was  $30.2 \pm 20.7$   $\mu$ U/ml compared to  $2.55 \pm 0.89$   $\mu$ U/ml in the control population ( $p < 0.01$ ). The greatest increase in insulin concentration was observed in the umbilical venous plasma. A twofold increase in fetal plasma glucose concentration was associated with a fifteenfold increase in fetal plasma insulin concentration. By contrast, a twofold increase in maternal glucose concentration was associated with only a sixfold increase in maternal plasma insulin concentration.

The mean body weights and sex ratios were not different between the two groups of animals (Table I). A



Table I. Characteristics of Groups 1 and 2

| Characteristic                                               | Group 1:<br>Control (8) | Group 2:<br>Treated (8) |
|--------------------------------------------------------------|-------------------------|-------------------------|
| Body weight (gm)                                             | 281.4 ± 71.9*           | 267.6 ± 51.3            |
| Male fetuses/female fetuses                                  | 3/5                     | 5/3                     |
| V <sub>max</sub> (total lung volume)                         | 21.7 ± 7.4              | 31.0 ± 4.7†             |
| V 15% D (residual lung volume at pressure of 15 cm of water) | 40.8 ± 15.8             | 39.4 ± 7.5              |
| Amniotic fluid L/S ratio                                     | 0.55 ± 0.21             | 0.49 ± 0.21             |
| Phosphatidylcholine/lung wet weight (μg/mg)‡                 | 2.62 ± 0.46             | 2.64 ± 0.58             |
| Phosphatidylcholine/DNA (μg/μg)‡                             | 0.36 ± 0.08             | 0.39 ± 0.12             |

\*Mean ± standard deviation.

†p < 0.01.

‡n = 7.

marked increase (43%) in the maximum lung volume of the treated animals was noted. However, those characteristics of increased surfactant activity measured in this study were not increased. The amniotic fluid L/S ratio, the residual deflation volume (at transpulmonary pressure of 30, 20, 15, 10, 5, and 0 cm of water), and the lung phosphatidylcholine concentrations (expressed per milligram of wet weight of lung or per microgram of DNA) were not different in the two groups of animals. However, when the fetal lung phosphatidylcholine concentrations were expressed as a percentage of total phospholipids (PC/PL) or as a ratio of phosphatidylcholine concentration to sphingomyelin concentration (PC/S), the amount of phosphatidylcholine was significantly increased in the treated animals (Fig. 3). The PC/PL in the treated animals (33.7% ± 1.7%, mean ± SEM) was higher than that of the control animals (29.0% ± 1.0%, p < 0.05). Also the PC/S of the treated animals (2.55 ± 0.18) was significantly greater than that of the control animals (2.18 ± 0.15, p < 0.025).

Both the fetal insulin and glucose concentrations were found to correlate significantly with the maximum lung volumes among the treated and untreated animals together (Table II). However, neither the fetal insulin concentrations nor the glucose concentrations correlated with the amniotic fluid L/S, the lung phosphatidylcholine concentrations expressed per milligram of lung or per microgram of DNA, or the residual lung volume on deflation at a transpulmonary pressure of 15 cm of water. (Since we have found the residual volume at a transpulmonary pressure of 15 cm of water (V 15% D) to be the most sensitive index of alveolar stability, we chose to evaluate correlations with this measurement.)<sup>12</sup> Correlations between fetal insulin and glucose were noted for the PC/PL values, but only fetal

Table II. Correlation coefficients in Groups 1 and 2

|                                                              | Fetal insulin           | Fetal glucose           |
|--------------------------------------------------------------|-------------------------|-------------------------|
| V <sub>max</sub> (total lung volume)                         | r = 0.62<br>(p < 0.01)  | r = 0.51<br>(p < 0.025) |
| V 15% D (residual lung volume at pressure of 15 cm of water) | r = 0.05<br>(NS)        | r = 0.05<br>(NS)        |
| Amniotic fluid L/S                                           | r = -0.04<br>(NS)       | r = 0.25<br>(NS)        |
| Phosphatidylcholine/mg lung                                  | r = 0.03<br>(NS)        | r = 0.06<br>(NS)        |
| Phosphatidylcholine/μg DNA                                   | r = 0.23<br>(NS)        | r = 0.22<br>(NS)        |
| PC/PL                                                        | r = 0.56<br>(p < 0.025) | r = 0.76<br>(p < 0.005) |
| PC/S                                                         | r = 0.30<br>(NS)        | r = 0.53<br>(p < 0.025) |

NS: Not significant.

glucose concentrations demonstrated a correlation with the PC/S values.

Comment

In the present study, we noted increases in maximum lung volume, associated with betamethasone administration, but failed to find changes in fetal lung surfactant, as measured by changes in amniotic fluid L/S ratios, increased alveolar stability, or lung phosphatidylcholine concentrations. The absence of these changes could be due to the associated hyperinsulinemia observed in this study. Other investigators have demonstrated that glucocorticoid-induced changes in lung function are consistent with increased surfactant production.<sup>1-3</sup> This discrepancy of results could be due to species differences or differences in the animal preparation concerning the method of glucocorticoid administration. In the majority of previous in vivo animal studies, glucocorticoids have been administered directly into the fetus.<sup>1-3</sup> Glucocorticoids administered to the adult are known to result in hyperglycemia and hyperinsulinemia apparently occurs secondary to this hyperglycemia. Maternal insulin is not thought to cross the placenta, but maternal glucose crosses by facilitated diffusion and could produce secondary hyperinsulinemia in the fetus.<sup>14</sup> Whether the administration of glucocorticoids directly to the fetus results in appreciable hyperinsulinemia is not known. A glucose infusion for 30 minutes in premature infants has been reported to result in a minimal insulin response.<sup>14</sup> This suggests that the degree and duration of fetal hyperglycemia are important in the type of insulin response that occurs. Not only the route of administration but also the potency and dosage of the glucocorticoid could have important relations to the extent of fetal hyperinsulinemia produced.

Unfortunately, previous *in vivo* studies demonstrating surfactant changes after glucocorticoids have not reported fetal insulin concentrations.<sup>1, 3</sup> We cannot conclude that insulin accounts for the different results between this study and previous studies.

Another possible explanation for the disagreement between our results and those of other studies is the difference in surfactant measurement. We place great importance on changes in alveolar deflation stability. We believe that in the absence of increased stability, functional increases in surfactant are not likely to be present.<sup>12, 15</sup> Other investigators have tended to rely on changes in lung phospholipid concentrations.

As a chemical index of lung surfactant concentration, we measured total phosphatidylcholine concentrations, which includes unsaturated and disaturated phosphatidylcholines. The disaturated phosphatidylcholines are thought to be the most important surface-active lipids<sup>1, 3</sup> and increases might not be detected by our analyses. However, in previous studies we reported significant correlations between total phosphatidylcholine concentrations and functional indices of lung surfactant, i.e., minimum extract surface tension values and lung deflation characteristics.<sup>12</sup> Therefore, total phosphatidylcholine concentrations appear to be a suitable biochemical marker of surfactant for the purposes of these studies.

As in previous investigations, we did not find that betamethasone treatment produced an increase in lung phosphatidylcholine concentrations.<sup>12</sup> However, among the treated fetuses, there were relative increases in phosphatidylcholine when expressed as a percentage of total phospholipids or as a ratio to lung sphingomyelin concentrations. Betamethasone treatment was associated with subtle alterations in lung lipid proportions, primarily due to slight reductions in total phospholipid and sphingomyelin concentrations. Whether the changes in PC/PL and PC/S have any functional significance remains to be determined. Their positive correlations with fetal insulin and glucose concentrations suggest synergistic relationships.

Of interest is the finding of fetal lung changes in

several animal preparations with induced diabetes. Epstein et al.<sup>16</sup> studied the fetuses of streptozotocin-treated rhesus monkeys and found an increased rate of choline incorporation into lecithin but no increase in lung lecithin concentrations. The studies on lung lecithin synthesis were done on lung slices and the amount of insulin present was not noted. Sosenko and co-workers<sup>17</sup> studied the fetuses of rabbits made diabetic with alloxan. They found functional evidence of less surfactant but no biochemical evidence and concluded that the surfactant in diabetic fetuses was qualitatively different from that in normal fetuses. Whether these chronic hyperinsulinemia circumstances have any relationship to the acute hyperinsulinemia of the present study is not known. If hyperinsulinemia does in fact impede the glucocorticoid action of increasing lung surfactant production, it will be important to identify the type and dosage of glucocorticoid least susceptible to this interaction, in order to provide maximal benefits to the fetus under treatment.

As in previous studies, we found that betamethasone administration was associated with a marked increase in total fetal lung volume.<sup>12, 15</sup> We believe that this increase in lung volume is mediated through betamethasone effects on lung connective tissues rather than through surfactant effects. Hyperinsulinemia may also bring about alterations in lung connective tissue, since it has been reported to affect collagen biosynthesis in skin fibroblasts.<sup>18</sup> Further studies are needed to delineate the causes of the marked increases in total lung volume.

The betamethasone-induced increases in maternal and fetal glucose and insulin values noted in this study are alarming. The implications that these effects may have with regard to immediate and long-term carbohydrate metabolism have been considered elsewhere.<sup>19</sup> Until further studies have documented the extent and consequences of these changes in the human, these potent glucocorticoids should not be used in the pregnant diabetic patient.

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# Control of mineral homeostasis during lactation: Interrelationships of 25-hydroxyvitamin D, 24,25-dihydroxyvitamin D, 1,25-dihydroxyvitamin D, parathyroid hormone, calcitonin, prolactin, and estradiol

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To further define control of mineral homeostasis during lactation, 28 lactating (L) and 20 nonlactating (NL) women were studied at 6 weeks post partum. Serum and urine calcium, magnesium, and phosphorus were normal and the same in both groups. 25-Hydroxyvitamin D (25-OHD) was the same (L,  $18.6 \pm 4.8$ ; NL,  $17.0 \pm 5$  ng/ml) in spite of a twofold higher intake of vitamin D in the lactating group. The serum fractions containing 24,25-dihydroxyvitamin D ( $24,25(\text{OH})_2\text{D}$ ) were lower than in nonpregnant adults in both groups but significantly lower ( $P < 0.05$ ) in lactating than in nonlactating women (L,  $1.67 \pm 1.7$ ; NL,  $2.46 \pm 1.3$  ng/ml). 1,25-Hydroxyvitamin D ( $1,25(\text{OH})_2\text{D}$ ) was normal in both groups (L,  $25.8 \pm 8.6$ ; NL,  $31.8 \pm 8.1$  pg/ml). Parathyroid hormone (PTH) was normal in both groups (L,  $5.6 \pm 2.0$ ; NL,  $7.2 \pm 1.9$   $\mu\text{l}$  Eq/ml), and calcitonin (HCT) was equally detectable. As expected, prolactin was higher in the lactating group (L,  $46 \pm 36$ ; NL,  $14.3 \pm 14.9$  ng/ml). Serum prolactin levels had no correlation with serum  $1,25(\text{OH})_2\text{D}$ . Estradiol was significantly ( $P < 0.02$ ) lower in lactating women (L,  $78 \pm 23$ ; NL,  $105 \pm 28$  pg/ml). One could speculate that the lower levels of estradiol in the presence of low  $24,25(\text{OH})_2\text{D}$  and normal HCT allow bone resorption to occur at a rate adequate to supply the mineral needs of lactation without elevations of either PTH or  $1,25(\text{OH})_2\text{D}$ . (AM. J. OBSTET. GYNECOL. 139:471, 1981.)

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DURING PREGNANCY the woman increases the intestinal absorption of calcium<sup>1</sup> and the renal reabsorption of calcium<sup>2</sup> to the point that she not only provides 25 gm of calcium to the growing fetus<sup>3</sup> but also actually increases her bone mass.<sup>4</sup> The vitamin D metabolites appear to play an important role in this process. The plasma fraction containing 24,25-dihydroxyvitamin D ( $24,25(\text{OH})_2\text{D}$ ) is decreased,<sup>5, 6</sup> whereas 1,25-dihydroxyvitamin D ( $1,25(\text{OH})_2\text{D}$ ) is increased.<sup>7-9</sup> This occurs in the face of modest increases in parathyroid hormone (PTH) which are still within the normal range<sup>10, 11</sup> and a normal serum phosphorus.<sup>10, 12, 13</sup> Several hormones which are known to increase the conversion of 25-hydroxyvitamin D (25-OHD) to  $1,25(\text{OH})_2\text{D}$  and decrease the production of  $24,25(\text{OH})_2\text{D}$



**Table I.** Serum and urine concentrations of minerals in lactating and nonlactating groups (Mean  $\pm$  SD)

|                    | <i>n</i> | <i>Calcium (mg/dl)</i> | <i>Magnesium (mEq/L)</i> | <i>Phosphorus (mg/dl)</i> |
|--------------------|----------|------------------------|--------------------------|---------------------------|
| <i>Serum:</i>      |          |                        |                          |                           |
| Lactating          | 29       | 9.1 $\pm$ 1.5          | 1.55 $\pm$ 0.27          | 3.8 $\pm$ 0.9             |
| Nonlactating       | 20       | 9.0 $\pm$ 1.1          | 1.56 $\pm$ 0.19          | 3.6 $\pm$ 0.5             |
| Pregnant           | 24       | 8.6 $\pm$ 0.75         | 1.25 $\pm$ 0.15          |                           |
| Normal women       | 27       | 9.2 $\pm$ 0.54         | 1.53 $\pm$ 0.15          | 3.6 $\pm$ 0.5             |
| <i>Spot urine:</i> |          |                        |                          |                           |
| Lactating          | 14       | 6.7 $\pm$ 6.7          | 3.8 $\pm$ 1.8            | 29.3 $\pm$ 19.2           |
| Nonlactating       | 7        | 4.3 $\pm$ 2.6          | 3.0 $\pm$ 1.5            | 27.8 $\pm$ 16.0           |

in animals have been postulated to modulate these changes: these hormones are prolactin,<sup>14</sup> estrogen,<sup>15, 16</sup> and placental lactogen.<sup>17</sup> Two studies have reported an elevation of calcitonin (HCT) during pregnancy,<sup>11, 18</sup> which was thought to protect the bone from the effects of the elevated 1,25(OH)<sub>2</sub>D, but three other studies have reported normal levels of calcitonin during pregnancy.<sup>5, 10, 19</sup>

During lactation, the maternal losses of calcium (~400 mg/day) and phosphorus (~200 mg/day) for the production of milk<sup>20</sup> are greater than fetal requirements. Intestinal calcium absorption and renal calcium reabsorption have not been studied during lactation. Resorption of bone (bone stored during pregnancy) occurs during lactation.<sup>4</sup> In rats, 1,25(OH)<sub>2</sub>D is increased during lactation,<sup>21</sup> and Kumar and associates<sup>9</sup> reported that 1,25(OH)<sub>2</sub>D was elevated in human beings during early lactation. However, the role of the vitamin D metabolites in human lactation past the immediate postpartum period has not been studied. Serum PTH levels during early lactation are not elevated,<sup>10, 22</sup> but Retallack and associates<sup>12</sup> found elevated PTH at 16 to 26 weeks' lactation. Rats are reported to have an elevated plasma HCT during lactation,<sup>23</sup> possibly to combat the known bone resorptive activities of elevated plasma 1,25(OH)<sub>2</sub>D. In human beings, one assay which showed plasma elevations of HCT during pregnancy showed much less elevation during lactation,<sup>18</sup> and another assay which found normal values during pregnancy found normal postpartum values in both lactating and nonlactating women.<sup>10</sup> Of the other serum hormones elevated during pregnancy, only prolactin remains elevated during lactation, and it remains elevated only for a few months even though nursing continues.<sup>24</sup> Probably of more significance than elevation of hormones is the depression of estrogen during nursing, which may permit increased bone resorption.<sup>25</sup>

Thus, in order to further evaluate the roles of the vitamin D metabolites and the hormonal milieu of human lactation on calcium and phosphorus homeostasis, samples of blood and urine were taken from lactating and postpartum control women for measure-

ments of 25-OHD, 24,25(OH)<sub>2</sub>D, 1,25(OH)<sub>2</sub>D, PTH, HCT, prolactin, estradiol, calcium, phosphorus, and magnesium.

### Methods

Blood was drawn and a spot urine was collected from 16 lactating and 13 nonlactating women during September and October of 1977. A second group of 12 lactating and seven nonlactating women were studied in February and March of 1978. The mean  $\pm$  SD weeks post partum was 5.8  $\pm$  1.5 and 6.3  $\pm$  2.3, respectively. Eighteen of 20 postpartum controls were between 4 and 8 weeks post partum, so that a portion of them had resumed menstrual cycles; however, no attempt was made to standardize the phase of the cycle to control for cyclic hormonal changes. The nursing women were somewhat older (30.1  $\pm$  4.0 versus 26.7  $\pm$  3.1) but of similar parity and race (89% versus 90% white). Mean  $\pm$  SD intake of vitamin D was twice as high in the lactating mothers since most had continued their prenatal vitamins, whereas the nonnursing women had not (4,478  $\pm$  1,846 versus 1,954  $\pm$  1,319 IU/week). Mean  $\pm$  SD hours outside were higher for both groups in the summer but did not differ between groups (lactating, 7.0  $\pm$  4.4 in the summer and 1.2  $\pm$  1.1 in the winter; nonlactating, 9.4  $\pm$  6.5 in the summer and 2.3  $\pm$  3.4 in the winter). During August and February, 27 women of childbearing age, who had not been pregnant and were not on oral contraceptives, served as controls for determinations of minerals. Pregnancy values for 25-OHD, 24,25(OH)<sub>2</sub>D, 1,25(OH)<sub>2</sub>D, and PTH are those previously published by the same assay methods on different subjects.<sup>5, 8, 23</sup>

Serum 25-OHD and 24,25(OH)<sub>2</sub>D were measured by the method previously described.<sup>26, 27</sup> Accurate estimations of 24,25(OH)<sub>2</sub>D appear to require multiple chromatographic isolation steps. The data reported here represent a single chromatographic step which may allow for contamination of 24,25(OH)<sub>2</sub>D by 25,26(OH)<sub>2</sub>D.<sup>28</sup> Since the subjects who received larger amounts of D<sub>2</sub> (lactating women) had lower apparent 24,25(OH)<sub>2</sub>D levels than nonlactating women, the re-

**Table II.** Serum hormones and vitamin D metabolites in lactating and nonlactating women (mean  $\pm$  SD)

|                                  | Lactating post partum (29) | Nonlactating post partum (20) | Normal women  | Term pregnancy                          |
|----------------------------------|----------------------------|-------------------------------|---------------|-----------------------------------------|
| 25-OHD (ng/ml)                   | 18.6 $\pm$ 4.8             | 17.0 $\pm$ 5                  | 24 $\pm$ 10   | 21 $\pm$ 1.8 <sup>5</sup> (n = 24)      |
| 1,25(OH) <sub>2</sub> D (pg/ml)  | 25.8 $\pm$ 8.7             | 31.8 $\pm$ 8.1                | 32 $\pm$ 7.5  | 73 $\pm$ 19 <sup>8</sup> (n = 5)        |
| 24,25(OH) <sub>2</sub> D (ng/ml) | 1.67 $\pm$ 1.7*            | 2.46 $\pm$ 1.3                | 3.9 $\pm$ 1.5 | 2.9 $\pm$ 1.3 <sup>5</sup> (n = 24)     |
| Estradiol (pg/ml)                | 78.1 $\pm$ 22.7†           | 105.0 $\pm$ 27.9              | 70 $\pm$ 180  | 14,070 $\pm$ 750 <sup>37</sup> (n = 27) |
| Prolactin (ng/ml)                | 46 $\pm$ 36*               | 14.3 $\pm$ 14.9               | 9.0 $\pm$ 3.2 | 251 $\pm$ 232 <sup>23</sup> (n = 19)    |
| PTH ( $\mu$ Eq/ml)               | 5.6 $\pm$ 2.0*             | 7.2 $\pm$ 1.9                 | 7.4 $\pm$ 2.7 | 5.1 $\pm$ 1.0 <sup>5</sup> (n = 10)‡    |

\*P < 0.05 lactating different from post partum controls.

†P < 0.02 lactating different from post partum controls.

‡Two to three hours post partum.

sults are acceptable for comparative purposes in this study. Only summer 24,25(OH)<sub>2</sub>D data are presented since these should be least affected by 25,26(OH)<sub>2</sub>D<sub>2</sub>. Measurement of 1,25(OH)<sub>2</sub>D was by radioreceptor assay<sup>8</sup> and was performed only on samples obtained in the winter. PTH was measured by an immunoassay which recognized the intact hormone and C-terminal fragments.<sup>28</sup> HCT was measured by a radioimmunoassay (sensitivity to 30 pg/ml).<sup>19</sup> Prolactin was measured by radioimmunoassay<sup>30</sup> in the core laboratories of Washington University, and estradiol was measured by radioimmunoassay.<sup>31</sup> Serum and urine calcium and magnesium were measured by atomic absorption spectrometry,<sup>32</sup> and phosphorus by a previously described technique.<sup>33</sup> Means and standard deviations were computed for each group, and comparisons were made by a nonpaired Student's t test, with a P value of less than 0.05 considered to be significant.

### Results

Serum and urine values for calcium, magnesium, and phosphorus (Table I) were the same in lactating and nonlactating groups and did not differ from those in nonpregnant controls. As indicated in Table II, prolactin was higher and estradiol was lower in the lactating women. PTH was normal in both lactating and postpartum control women, and, indeed, the mean PTH of the lactating women was significantly lower statistically (P < 0.05) than that of the control women. There was no increase in detectability of HCT in the lactating women (2/17 lactating, 6/16 controls). Values of serum 25-OHD were similar in lactating and nonlactating groups in spite of the greater intake of vitamin D by the lactating group. In both lactating and nonlactating groups, 24,25(OH)<sub>2</sub>D was lower than normal and was significantly lower in the lactating women than in the control group (P < 0.05). In both the lactating and nonlactating groups, 1,25(OH)<sub>2</sub>D was within the normal range.

In the lactating group, there were correlations of serum calcium and phosphorus (r = 0.65, P < 0.01),

**Table III.** Comparison of hormonal changes during pregnancy and lactation

| Hormone                  | Pregnancy   | Lactation |
|--------------------------|-------------|-----------|
| Prolactin                | ↑↑          | ↑         |
| Estradiol                | ↑↑          | ↓         |
| 25-OHD                   | Normal      | Normal    |
| 24,25(OH) <sub>2</sub> D | ↓           | ↓↓        |
| 1,25(OH) <sub>2</sub> D  | ↑           | Normal    |
| PTH                      | Normal or ↑ | Normal    |
| HCT                      | Normal or ↑ | Normal    |

calcium and magnesium (r = 0.89, P < 0.01), and magnesium and phosphorus (r = 0.74, P < 0.01). In the nonlactating group, serum calcium and phosphorus were not correlated (r = 0.14, NS). However, calcium and magnesium maintained a correlation (r = 0.71, P < 0.01) similar to that seen in pregnancy (r = 0.60, P < 0.01). Positive correlations of calcium, magnesium, and phosphorus could also be seen in spot urines from lactating women but not in those from nonlactating controls. The correlation of serum phosphorus and urine phosphorus in lactating women was r = 0.71, P < 0.01, whereas there was no correlation in controls (r = 0.01). Such correlations are not seen in normal adult populations.<sup>34</sup>

### Comment

The best hypothesis for the strong positive correlations of serum calcium, magnesium, and phosphorus and, also, urine calcium, magnesium, and phosphorus seen in the lactating women is an active mobilization of minerals from resorbed bone or some degree of intestinal hyperabsorption. Similar correlations were not seen in the control postpartum women and are not seen in normal women. This mobilization or absorption of minerals is of such a degree that, in spite of lactational drains, the serum and urine calcium, magnesium, and phosphorus excretions are similar in lactating and nonlactating postpartum women. Previous studies have shown that bone mass increases during pregnancy and decreases during lactation.<sup>4</sup>



The stimulus to bone resorption during lactation is probably a combination of permissive and active factors. Post partum, serum estrogens fall rapidly to levels below those normally seen in the nonpregnant state.<sup>35</sup> Lactation maintains these subnormal concentrations of estrogens, and ovulation may be prevented for many months, depending upon the degree of lactation; without lactation, estrogens return to normal and ovulation resumes.<sup>25</sup> By mechanisms which are unclear, estrogens block bone resorption,<sup>36</sup> and the very high levels seen in pregnancy<sup>37</sup> may play an important role in bone accretion during pregnancy. Post partum, the rapid fall in estrogens removes the block, and bone resorption proceeds. Lactation inhibits the return of the normal regulation of resorption by estrogens. Calcitonin (HCT) also blocks bone resorption. Although our own data in two successive studies<sup>5, 19</sup> clearly do not show an elevation of HCT during pregnancy, the radioimmunoassay used by Stevenson and associates<sup>18</sup> showed an elevation of HCT in sera from pregnant subjects. With that assay, levels of HCT during lactation are markedly less than during pregnancy, although still slightly higher than in nonpregnant controls.<sup>18</sup> In this study, the levels of HCT in lactating women were not higher than those seen in postpartum controls.

With a decrease in blocking factors, smaller amounts of active resorbing factors, PTH and  $1,25(\text{OH})_2\text{D}$ , may be needed. Indeed, PTH was normal in lactating women and slightly lower than in control women in both summer and winter groups;  $1,25(\text{OH})_2\text{D}$  was also low normal in the lactating women and not statistically different from that in postpartum controls. This is in contrast to the findings in rats, in which both HCT and  $1,25(\text{OH})_2\text{D}$  were elevated during lactation.<sup>8, 23, 38</sup> Kumar and associates<sup>9</sup> reported an elevation of  $1,25(\text{OH})_2\text{D}$  during lactation; however, this conclusion was based on samples from six women in the postpartum period (exact days post partum not given). The mean plasma level in lactating women was already decreasing from the values during their pregnancies. Lund and associates,<sup>39</sup> in a preliminary report, showed a rapid fall in serum  $1,25(\text{OH})_2\text{D}$  post partum, with some slight continued elevation at 6 days post partum.<sup>39</sup> Their mean level at 30 days post partum was 37, with their adult normal being  $33 \pm 15$  pg/ml.<sup>39</sup> Our data at 6 weeks post partum confirm that the residual effects of the  $1,25(\text{OH})_2\text{D}$  elevations of pregnancy are relatively short-lived, and that lactation does not increase the levels of  $1,25(\text{OH})_2\text{D}$  over control postpartum serum levels.

In spite of a twofold higher intake of vitamin D by the lactating women (640 versus 280 IU/day), the serum concentrations of 25-OHD were the same in lac-

tating and control women. The predominant source of vitamin D is from skin conversion, and exposures to the sun were similar in lactating and nonlactating women. In a previous study of pregnant women during the summer months, no relationship between intake of vitamin D and serum concentration of 25-OHD could be seen. However, in the winter, a weak correlation did exist.<sup>40</sup> Thus, given the limitations of dietary and sun-exposure histories, the failure to see higher serum levels of 25-OHD in the lactating women, especially in the winter, could represent, in part, a loss of vitamin D as vitamin D, 25-OHD, and  $24,25(\text{OH})_2\text{D}$  into the milk.<sup>41, 42</sup>

Both pregnant<sup>5, 6</sup> and lactating women had lower  $24,25(\text{OH})_2\text{D}$  than did nonpregnant women. At 6 weeks post partum, control women still had a depression of  $24,25(\text{OH})_2\text{D}$ ; however, this was significantly less than in the lactating women. In rats,  $24,25(\text{OH})_2\text{D}$  was also decreased in both pregnancy and lactation.<sup>38</sup> Furthermore, an asynchrony of changes in  $24,25(\text{OH})_2\text{D}$  and  $1,25(\text{OH})_2\text{D}$  was noticed in the rats, in that  $24,25(\text{OH})_2\text{D}$  fell during pregnancy prior to the rise in  $1,25(\text{OH})_2\text{D}$ .<sup>38</sup> This would be consistent with an early rise in prolactin and a later rise in PTH. In our lactating human subjects,  $24,25(\text{OH})_2\text{D}$  was depressed but  $1,25(\text{OH})_2\text{D}$  remained normal. Again, this asynchrony may be explained by the elevated prolactin but normal PTH. The only hormonal change common to pregnancy and lactation was the elevation of prolactin. Although animal data suggested an effect of prolactin on elevating  $1,25(\text{OH})_2\text{D}$  and decreasing  $24,25(\text{OH})_2\text{D}$ ,<sup>14</sup> data in human subjects have failed to confirm an elevation of  $1,25(\text{OH})_2\text{D}$  in patients with hyperprolactinemia,<sup>43, 44</sup> and, indeed, our lactating women had normal serum concentrations of  $1,25(\text{OH})_2\text{D}$ .  $24,25(\text{OH})_2\text{D}$  has not been studied in human beings with hyperprolactinemia independent of pregnancy or lactation. In the lactating women, there was also no correlation between  $1,25(\text{OH})_2\text{D}$  and prolactin or between  $1,25(\text{OH})_2\text{D}$  and PTH. On the other hand, there was a suggestive weak inverse correlation between  $24,25(\text{OH})_2\text{D}$  and prolactin in the lactating women ( $r = -0.46$ ,  $0.05 < P < 0.1$ ). It has been suggested that  $24,25(\text{OH})_2\text{D}$  is important for chondrocyte growth<sup>45</sup> and matrix formation<sup>46</sup> and, thus, may be important for bone accretion. A low  $24,25(\text{OH})_2\text{D}$  might be preferable for conditions which favor bone resorption.

Gastrointestinal absorption of calcium remains slightly elevated for a few weeks post partum but has not been studied in actively lactating women.<sup>47</sup> Since the acute effect of estrogens is inhibition of absorption of calcium, usually later compensated for by elevations of PTH and  $1,25(\text{OH})_2\text{D}$ ,<sup>48</sup> lower levels of estrogens could possibly

increase gastrointestinal absorption in the face of normal PTH and  $1,25(\text{OH})_2\text{D}$ .

A summary and comparison of mineral homeostasis during pregnancy and lactation can be seen in Table III. In the short run, for several months, the hormonal changes of lactation, i.e., decreased estradiol and possibly the elevation of prolactin, decreased  $24,25(\text{OH})_2\text{D}$ , and normal HCT, maintain normal serum calcium and phosphorus while providing for the production of milk without secondary hyperparathyroid-

ism and/or elevations of  $1,25(\text{OH})_2\text{D}$ . After several months, when prolactin falls,<sup>24</sup> and if estrogens increase and menses resume, this balance may not persist without the secondary hyperparathyroidism noticed by Retallack and associates<sup>12</sup> at 16 to 26 weeks' lactation. This study was performed at 6 weeks post partum, not because of physiologic considerations, but because that is the customary timing of postpartum visits. Serial studies from pregnancy throughout prolonged lactation are needed and are now in progress.

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# Desoxycorticosterone in normal pregnancy

## III. Evidence of a fetal source of desoxycorticosterone

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The source of the markedly increased secretion of desoxycorticosterone (DOC) in pregnancy has not been precisely defined. Earlier studies indicated that elevated DOC does not arise from the maternal adrenal glands. The previously observed steep gradient between fetal and maternal DOC and DOC sulfate concentrations seemed to point to a fetal source. A recent study suggests that DOC may also be derived from extra-adrenal conversion of maternal progesterone. The present investigation documents a lack of synchrony between maternal diurnal plasma cortisol and DOC patterns. Not only were the previous observations of steep gradients of DOC and DOC sulfate between fetal and maternal circulations confirmed, but also the finding of extremely high urinary excretion of DOC sulfate indicated that the sulfurylated steroid passes across the placenta. A direct linear correlation was noticed between cortisol and DOC in amniotic fluid. These observations suggest that the increased DOC arises from within the fetoplacental unit but do not rule out a maternal source. (AM. J. OBSTET. GYNECOL. 139:477, 1981.)

THE SOURCE OF the markedly increased desoxycorticosterone (DOC) secreted in the third trimester of normal pregnancy has not been localized precisely. Previous studies in third-trimester gravid women showed that plasma DOC is unresponsive not only to stimulation by adrenocorticotrophic hormone (ACTH) and suppression by dexamethasone, but also to variations in the intake of salt.<sup>1-3</sup> These results suggested that the increased DOC secreted in late pregnancy does not arise from the maternal adrenal glands. On the basis of observations that concentrations of DOC and DOC sulfate (an inactive DOC metabolite) are much higher in mixed cord blood than in maternal venous blood, the assumption

was made that the fetoplacental unit is the source of the increased DOC.<sup>3, 4</sup> However, these findings do not necessarily exclude the possibility that there may also be maternal production of DOC. Recently, Winkel and co-workers<sup>5</sup> presented evidence that DOC is produced within the maternal compartment by the extra-adrenal 21-hydroxylation of progesterone. Described in the present report are additional experiments that were performed to study the regulation, and to define further the source(s), of the increased DOC secreted in pregnancy.

### Methods

Concentrations of DOC and cortisol were measured in samples of venous blood obtained from seven normal third-trimester women at 2 hour intervals during a 24 hour period of study beginning at 0800 hours. Volunteers, who ranged in age from 17 to 21 years, were hospitalized at least 48 hours prior to the start of the study. The subjects were given a regular hospital diet and remained supine in bed during the study period, except for brief interruptions while eating or when using the bathroom. The method for obtaining multiple samples of blood, with minimal disturbance of the subjects, was described previously.<sup>6</sup>

Concentrations of DOC and cortisol were measured in samples of amniotic fluid obtained from 22 women

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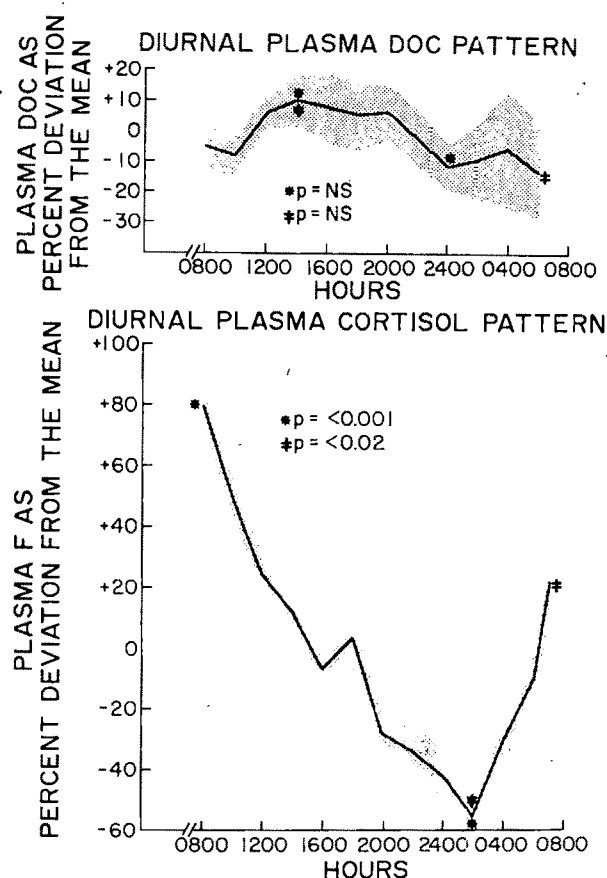


Fig. 1. Diurnal patterns of plasma concentrations of DOC and cortisol (F) in seven third-trimester pregnant women. Each point represents the mean derived value for that hour  $\pm$  SEM.

whose duration of gestation ranged from 34 to 40 weeks at the time of diagnostic amniocentesis. This procedure was performed in 18 women prior to elective repeat cesarean section and in four women with premature labor who had received dexamethasone, 12 mg orally, less than 24 hours previously in order to prevent the fetal respiratory distress syndrome.

Plasma concentrations of DOC and DOC sulfate were measured in mixed umbilical cord blood from nine normal term neonates and in peripheral venous blood from nine normal gravid women obtained at the time of uncomplicated vaginal delivery, and also in peripheral venous blood from nine normal nonpregnant women. DOC was also determined in mixed umbilical cord blood from four term neonates delivered by cesarean section. In addition, excretion rates of DOC and DOC sulfate were measured in 24 hour collections of urine obtained from nine ambulatory third-trimester gravid women and from nine nonpregnant women.

In all of the above-mentioned experiments, blood was withdrawn into plastic syringes and transferred

immediately into heparin-containing tubes. Plasma was separated by centrifugation within 1 hour. Biologic specimens were stored at  $-20^{\circ}\text{C}$  for subsequent steroid assays.

This study was approved by the Committee for the Protection of Human Subjects. Informed consent was obtained from volunteers who agreed to participate after being provided with a full explanation of the purpose of the study and the experimental procedure that would be performed.

**Corticosteroid measurements.** DOC in plasma, amniotic fluid, and urine was measured by radioimmunoassay, after extraction, acetylation, and descending paper chromatography, as reported earlier.<sup>1</sup> DOC sulfate remaining in the samples of extracted plasma and urine was solvolyzed by overnight incubation in acidic ethyl acetate, acetylated, chromatographed, and assayed as DOCA with the use of the DOC antibody.<sup>1, 7</sup> Validation of the specificity of the DOC sulfate assay was accomplished as follows. Of the known steroids that might be present in interfering amounts in our samples, only progesterone and testosterone cross-reacted to an appreciable degree with the antibody used. These steroids were eliminated by chromatography after acetylation. Parallelism studies indicated that chromatographed, acetylated extracts of maternal plasma or urine or of mixed umbilical cord blood did not contain significant amounts of unknown steroids that would cross-react with the antibody. After logit/log transformation, DOCA standard curves with and without added extract had virtually identical slopes.

Cortisol in plasma and amniotic fluid was measured by radioimmunoassay as previously reported.<sup>8</sup> After the samples had been washed with hexane-ethyl acetate, to remove progestins, plasma cortisol and "total amniotic fluid cortisol" were assayed directly in the aqueous phase. "Total amniotic fluid cortisol" measurements include unconjugated cortisol and also water-soluble cortisol metabolites which cross-react with the cortisol antibody. Unconjugated amniotic fluid cortisol was determined in the methylene chloride extract of the aqueous phase.

Results are expressed as the mean  $\pm$  the standard error of the mean. The significance of differences in corticosteroid concentrations was determined by the Student's *t* test for paired and unpaired data, as applicable.

**Reagents.** The reagents 1,2-<sup>3</sup>H-DOC and 1,2-<sup>3</sup>H-hydrocortisone (specific activities 50 Ci/mmol) were obtained from New England Nuclear, Boston, Massachusetts. Labeled steroids were at least 97% pure as determined by paper chromatography. Dextran No. D-4751 was supplied by Sigma Chemical Company, St.

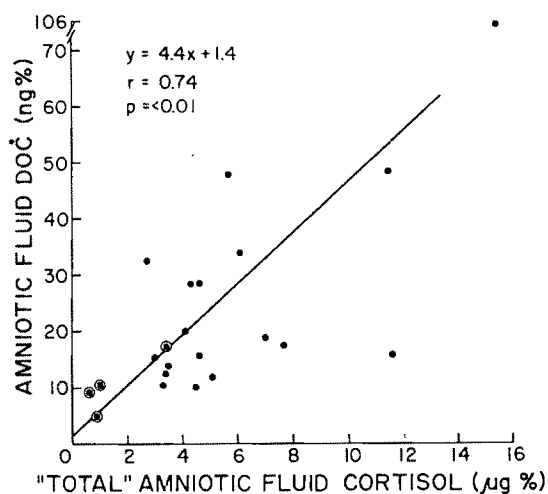


**Fig. 2.** Correlation of DOC and "total" cortisol in amniotic fluid from 22 third-trimester pregnant women. Concentric circles indicate prior treatment with dexamethasone.

Louis, Missouri; and carbon decolorizing alkaline Norit-A was obtained from Fisher Scientific Company, Fair Lawn, New Jersey. Antibodies used for radioimmunoassay were purchased from Endocrine Science Laboratories, Tarzana, California. All chemicals were analytical grade.

## Results

**Diurnal patterns of plasma DOC and cortisol in third-trimester pregnant women.** In Fig. 1 the percent deviation of plasma levels of DOC and cortisol from the 24-hour mean value of each steroid is plotted against the time of blood withdrawal. Maternal concentrations of cortisol followed a nyctohemeral pattern similar to that observed in the nonpregnant state, with levels being highest in midmorning and reaching a nadir shortly after midnight. Mean plasma cortisol was  $37.9 \pm 2.4$   $\mu\text{g}/100$  ml at 0800 hours and fell to  $12.3 \pm 2.1$   $\mu\text{g}/100$  ml at 2400 hours ( $p < 0.001$ ). Maternal plasma DOC levels in contrast failed to show a significant diurnal variation. The slight fluctuations of DOC were asynchronous with the distinct circadian cortisol rhythm. Mean plasma DOC was  $43.4 \pm 6.6$  ng/100 ml at 0800 hours and  $42.9 \pm 9.2$  ng/100 ml at 2400 hours.

**Correlation of DOC with total and unconjugated cortisol in amniotic fluid.** Fig. 2 shows concentrations of DOC plotted against levels of total cortisol measured in samples of amniotic fluid from 22 third-trimester pregnant women; four of these had been treated with dexamethasone. DOC ranged from 4.9 to 106 ng/100 ml (mean  $23.6 \pm 4.6$  ng/100 ml), and total cortisol from 0.6 to 15.4  $\mu\text{g}/100$  ml (mean  $5.1 \pm 0.8$   $\mu\text{g}/100$  ml). The direct linear correlation between DOC and

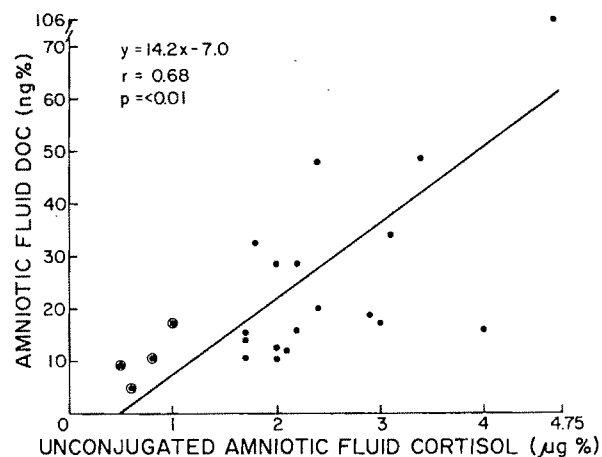


**Fig. 3.** Correlation of DOC and unconjugated cortisol in amniotic fluid from 22 third-trimester pregnant women. Concentric circles indicate prior treatment with dexamethasone.

total cortisol in amniotic fluid was significant ( $r = 0.74$ ,  $p < 0.01$ ). The relationship of concentrations of DOC to levels of unconjugated cortisol in the same 22 samples of amniotic fluid is shown in Fig. 3. Unconjugated cortisol ranged from 0.5 to 4.7  $\mu\text{g}/100$  ml (mean  $2.2 \pm 0.2$   $\mu\text{g}/100$  ml). The direct linear correlation between DOC and unconjugated cortisol in amniotic fluid was significant ( $r = 0.68$ ,  $p < 0.01$ ). Concentrations of DOC and cortisol were depressed appreciably in samples from women treated with dexamethasone. After deletion of values measured in dexamethasone-treated gravid women, the correlation between total cortisol and DOC and between unconjugated cortisol and DOC in the remaining 18 samples of amniotic fluid continued to be significant ( $r = 0.71$ ,  $p < 0.01$ ;  $r = 0.68$ ,  $p < 0.01$ ).

**Plasma DOC and DOC sulfate concentrations in mixed cord blood from normal neonates and in peripheral venous blood from term gravid women and nonpregnant women.** Fig. 4 shows the marked increase in the concentrations of both steroids in third-trimester term gravid women as compared to nonpregnant subjects. Mean plasma DOC was  $78.1 \pm 19.2$  ng/100 ml in pregnant women and  $10.5 \pm 2.0$  ng/100 ml in nonpregnant women. The corresponding mean levels of DOC sulfate were  $44.0 \pm 7.4$  ng/100 ml in gravid women and undetectably low in nonpregnant controls. Mean DOC and DOC sulfate in cord blood at the time of vaginal delivery were  $166 \pm 6$  and  $553 \pm 72$  ng/100 ml, resulting in concentration gradients of 2.1:1 and 12.6:1, respectively, between neonatal and maternal levels. The mean concentration of DOC in mixed cord blood of term neonates delivered by cesarean section was  $161 \pm 14.5$  ng/100 ml, which is not significantly



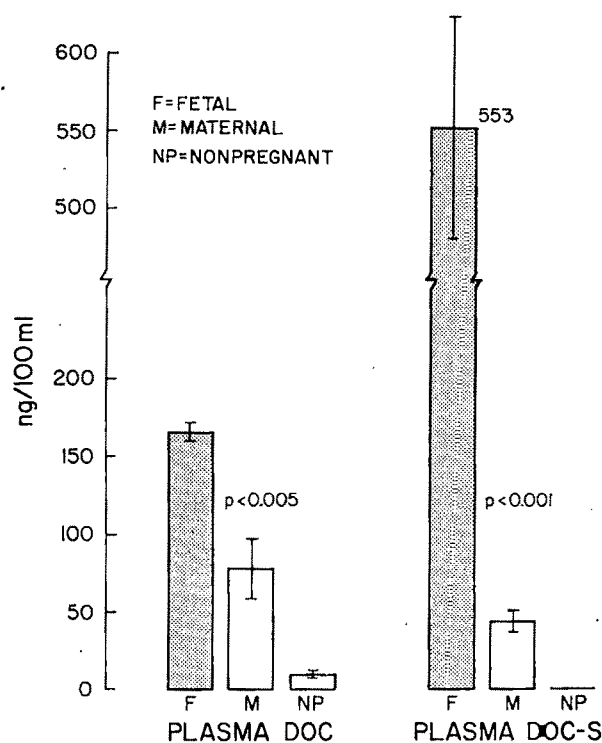


Fig. 4. Mean plasma concentrations of DOC and DOC sulfate (DOC-S) in neonates (fetal), term gravid women (maternal), and nonpregnant control subjects. Nine samples were measured in each group.

different from the value measured at the time of spontaneous vaginal delivery.

**Urinary excretion rates of DOC and DOC sulfate in pregnant and nonpregnant women.** The 24-hour urinary excretion rates of DOC and DOC sulfate are compared in Fig. 5. Mean urinary DOC excretion was  $1.1 \pm 0.2 \mu\text{g}/24$  hours in pregnant women and  $0.09 \pm 0.01 \mu\text{g}/24$  hours in nonpregnant women. The corresponding measurements of mean DOC sulfate excretion were  $2.9 \pm 0.6$  and  $0.4 \pm 0.1 \mu\text{g}/24$  hours. In gravid women, the urinary excretion rate of DOC was increased elevenfold above the nonpregnant mean value, whereas that of DOC sulfate was elevated 7.3-fold.

#### Comment

In normal nonpregnant individuals, the secretion of DOC increases in response to stimulation by ACTH and is depressed by dexamethasone, but is not directly affected by factors that influence the secretion of aldosterone.<sup>9</sup> Thus, it has been assumed that the small amount of DOC secreted in normal nonpregnant women is mainly a byproduct of cortisol production, and, accordingly, that it is dependent on ACTH. However, when studied in normal third-trimester pregnant

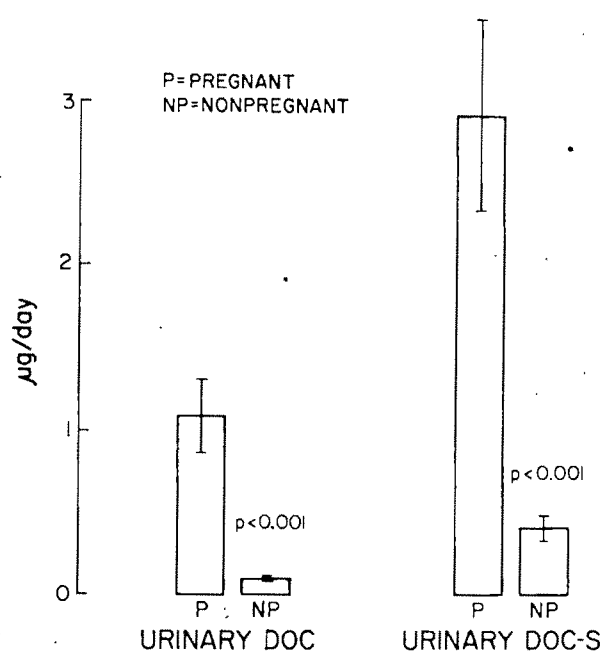


Fig. 5. Mean 24-hour urinary excretion rates of DOC and DOC sulfate in nine third-trimester pregnant women and nine nonpregnant control subjects.

women, the markedly elevated levels of plasma DOC not only were unresponsive to changes in the intake of salt, but, surprisingly, also failed to respond to either stimulation by ACTH or suppression by dexamethasone.<sup>1-3</sup> The observation that the secretion of DOC was not suppressed by the administration of dexamethasone or during chronic loading with salt suggested that the increased DOC does not arise from either glucocorticoid or mineralocorticoid pathways of the maternal adrenal glands. The lack of synchrony that was observed in the present study between circadian patterns of plasma cortisol and the insignificant fluctuations of plasma DOC in third-trimester gravid women strongly supports the proposition that increased secretion of DOC is not a byproduct of maternal ACTH-dependent adrenal secretory activity.

The much higher concentrations of DOC found in mixed cord blood than in maternal venous blood suggest that the fetoplacental unit could be a source of the increased DOC secreted in pregnancy. The close similarity between concentrations of DOC in the cord blood of neonates delivered either spontaneously or by cesarean section suggests that the markedly elevated levels in the fetus are not related to the stress of vaginal delivery. Since DOC is bound avidly to corticosteroid-binding globulin (CBG), it must be assumed that the "effective" fetal-maternal gradient between plasma concentrations of free DOC would be even greater

than that indicated by the marked differences in the total concentrations measured in the current study, because CBG levels are negligible in fetal blood. Thus, to whatever extent transplacental passage of DOC might occur, the extremely steep fetal-maternal gradient would favor transfer from the fetus to the mother. Since cortisol in amniotic fluid presumably reflects fetal production, the direct correlation observed between concentrations of DOC and both total and unconjugated cortisol in amniotic fluid, as well as the apparent depression of the concentration of these corticoids that was observed after administration of large pharmacologic doses of dexamethasone, points to a common biosynthetic pathway within the fetoplacental unit.<sup>10</sup> Since DOC and total or unconjugated cortisol levels were also significantly correlated in samples of amniotic fluid from gravid women who had clude that, in the nonsuppressed state, fetal DOC and cortisol in amniotic fluid are regulated by the same mechanism. The failure of the maternal plasma level of DOC to rise in response to administered ACTH should not be taken as evidence that the fetal adrenal glands are not involved in the increased production of DOC, since ACTH does not cross the placental barrier, but it does suggest that the secretion of DOC by the maternal adrenal glands is unresponsive to this stimulus.<sup>11</sup>

In keeping with previous reports, plasma concentrations of DOC sulfate in pregnancy are markedly elevated above nongravid levels, and are much greater in the fetus than in the mother.<sup>4</sup> Since sulfurylation of steroids presumably occurs mainly within the fetoplacental unit, the extremely high rate of excretion of DOC sulfate observed in the pregnant women, which is contrary to results of an earlier study by others, indicates that there is substantial passage of the sulfurylated steroid across the placenta to the maternal circulation.<sup>4, 12</sup> Despite the very high fetal levels, it is unlikely that DOC sulfate could be an indirect source of maternal DOC, because steroids of the pregn-4-ene-21-yl sulfate series, such as DOC sulfate, unlike estrogen sulfates, are not hydrolyzed by placental steroid 3-sulfatase.<sup>13</sup> Since DOC sulfate has been assumed to be an inactive DOC metabolite, it is tempting to speculate that the very high concentrations of DOC sulfate found in cord blood reflect a protective mechanism modulating the quantity of active DOC contributed to the maternal circulation by the fetoplacental unit.<sup>14</sup>

The above-mentioned findings do not necessarily exclude the possibility that there also may be maternal production of DOC. Winkel and co-workers<sup>5</sup> presented convincing evidence that a substantial amount of DOC is derived from 21-hydroxylation of circulating progesterone. Such extra-adrenal production of DOC would account for the observed lack of responsiveness of maternal plasma DOC to physiologic maneuvers that normally affect adrenocortical secretory activity. However, there still is no apparent explanation for the observed failure of DOC secretion to increase when the maternal adrenal glands are stimulated by the administration of ACTH, whereas the cortisol response to this stimulus is exaggerated compared to that in nongravid women.<sup>1, 15</sup> In accordance with recent findings by Winkel and associates,<sup>16</sup> it is possible that the peripheral conversion of progesterone to DOC observed in pregnancy is so substantial that the added response of the maternal adrenal glands to ACTH is relatively insignificant and thus evades detection.

Regardless of the source, it is apparent that large amounts of DOC are produced during pregnancy which are not governed by factors that normally regulate adrenocortical function. The markedly increased rate of urinary excretion of DOC observed in this study provides additional evidence that the plasma *free* DOC concentration is elevated above normal, since the quantity of unconjugated free steroid excreted in the urine is directly related to the circulating level of unbound steroid.<sup>17</sup> The possible role that such high levels of biologically active DOC might play in the pathogenesis of hypertensive disorders of pregnancy has not been fully explored. The resultant increment in mineralocorticoid activity, which seems to be well tolerated in normal pregnancy, might begin to express itself in abnormal circumstances. Accordingly, with the development of pre-eclampsia, aldosterone is depressed, but nonsuppressible DOC, although reported in one early study to be no higher than in normotensive gravid women, might then be excessive relative to the existing state of sodium balance.<sup>3</sup>

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# An increase in catecholamines and metabolites in the amniotic fluid compartment from middle to late gestation

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By means of a modified radioenzymatic assay, simultaneous determinations of the parent catecholamines, epinephrine (E), norepinephrine (NE), and dopamine (DA), and their deaminated metabolites, 3,4-dihydroxyphenylacetic acid (DOPAC) and 3,4-dihydroxyphenylglycol (DOPEG), were made in amniotic fluid obtained during the second (N = 44) and third (N = 20) trimesters of normal pregnancies. Significant positive correlations were noted between gestational age and concentrations of E, NE, DA, DOPAC, and DOPEG in amniotic fluid. These findings provide evidence of progressive fetal adrenergic maturation through pregnancy. In addition, our data suggest a parallel maturational event in the central nervous and peripheral catecholamine systems of the fetus, since DOPAC and DOPEG are more representative of catecholamine neuronal activity in the brain. (AM. J. OBSTET. GYNECOL. 139:483, 1981.)

COMPELLING EVIDENCE indicates that catecholamines in amniotic fluid are derived principally from the fetal compartment.<sup>1-3</sup> Therefore, changes in the concentrations of catecholamines in the amniotic fluid compartment during the course of gestation may reflect progressive maturation of the fetal adrenergic system or fetal adrenergic response to neurogenic stimuli.

Several studies indicate that a specific metabolite

of dopamine (DA), 3,4-dihydroxyphenylacetic acid (DOPAC),<sup>4</sup> and the alcohol metabolites of norepinephrine (NE), 3,4-dihydroxyphenylglycol (DOPEG) and 3-methoxy-4-hydroxyphenylethylglycol (MOPEG),<sup>4-6</sup> may be more representative of central nervous system (CNS) adrenergic activity than either the parent catecholamines or other metabolites which reflect primarily peripheral sympathetic activity.

Concentrations of E,<sup>3, 7-9</sup> NE,<sup>3, 7-10</sup> DA,<sup>10</sup> and various catecholamine metabolites<sup>11-13</sup> in amniotic fluid have been reported, but simultaneous measurement in the amniotic fluid of both parent catecholamines and CNS-specific metabolites has not been made. By means of a modified radioenzymatic assay,<sup>14</sup> concentrations of the parent catecholamines and their CNS-specific metabolites, DOPAC and DOPEG, were examined in 64 samples of amniotic fluid from uncomplicated pregnancies. Quantitative changes in these amniotic fluid amines between mid and late gestation were analyzed.

## Material and methods

Samples of amniotic fluid were obtained from 64 women, 44 of whom were 16 to 24 weeks' gestation (second trimester) and 20 of whom were 36 to 42 weeks' gestation (third trimester). Indications for amniocentesis were genetic counseling, elective abortion,

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**Table I.** Concentrations of catecholamines and their metabolites in amniotic fluid (all values are expressed as pg/ml)

|                | Second trimester (n = 44) |     |            | Third trimester (n = 20) |       |              |
|----------------|---------------------------|-----|------------|--------------------------|-------|--------------|
|                | Mean                      | SEM | Range      | Mean                     | SEM   | Range        |
| Dopamine       | 880                       | 98  | <10-2,557  | 1,362                    | 263   | <10-4,286    |
| DOPAC          | 7,780                     | 720 | 200-21,100 | 9,900                    | 1,080 | 1,400-18,100 |
| Epinephrine    | 449                       | 52  | 8-1,460    | 800                      | 149   | <2-1,299     |
| Norepinephrine | 152                       | 21  | 5-845      | 386                      | 63    | 82-1,148     |
| DOPEG          | 1,015                     | 78  | 270-2,500  | 1,446                    | 165   | 610-2,000    |

and determination of fetal lung maturity prior to repeat cesarean section. Specifically excluded were patients in labor and those with ruptured membranes, with otherwise complicated pregnancies, with fetal distress evident by antepartum or intrapartum electronic monitoring, and with a depressed or abnormal infant at birth. In none of the patients who underwent elective abortion was there any evident fetal or placental abnormality at delivery. Ultrasound determination at the time of amniocentesis revealed a normal amount of amniotic fluid. The women were receiving no medications other than prenatal vitamin and iron supplementation. Patients in the second and third trimesters did not differ significantly in terms of usage of tobacco and caffeine. Gestational age was calculated from the last menstrual period and correlated with fundal height, quickening, appearance of fetal heart tones, ultrasound, and neurological assessment at delivery.

Amniotic fluid obtained by transabdominal amniocentesis was collected in cold plastic tubes to which dithiothreitol, a reducing agent, had been added as a preservative. The specimens were immediately centrifuged at 2,500 rpm and 4° C for 15 minutes. The supernatant was then pipetted into cold plastic tubes and stored at -20° C up to 6 months until assay. Experiments were done which showed that the levels of catecholamines and metabolites were stable under these conditions of collection.

For the measurement of catecholamines and metabolites in amniotic fluid, a previously reported assay<sup>14</sup> was modified as follows. Aliquots (0.2 ml) of amniotic fluid were radiomethylated with catechol-O-methyltransferase<sup>15</sup> in the presence of 1.6  $\mu$ M <sup>3</sup>H-S-adenosylmethionine (5 to 15 Ci/mmol, New England Nuclear), 0.3M Tris, 30 mM MgCl<sub>2</sub>, 10 mM ethyleneglutamine-tetraacetic acid, and 0.1 mM dithiothreitol (pH 8.6) for 60 min at 37° C. The reaction was terminated by the addition of 0.2 ml of 1M Na<sub>3</sub>BO<sub>3</sub> (pH 11). The parent catecholamines and DOPEG were extracted into 2 ml of toluene/isoamyl alcohol (3:2) and subsequently back-extracted into 0.2 ml of 0.1M acetic acid containing 25  $\mu$ g each of 3-methoxytyramine, normetaneph-

rine, metanephine, and 3-methoxy-4-hydroxyphenylethylglycol as a cold carrier. After the addition of 0.2 ml of 100% ethanol, the samples were evaporated under a stream of N<sub>2</sub> at 45° C. The samples were applied to silica gel 60-F254 thin-layer chromatography (TLC) plates (EM Laboratories) which had been impregnated with sodium tetraborate by prewashing with a 0.1M solution. The plates were developed for 90 minutes with t-amyl alcohol/benzene/40% CH<sub>3</sub>NH<sub>2</sub>/CH<sub>3</sub>OH (6:2:3:1). The spots were visualized under short-wave ultraviolet irradiation, cut into scintillation vials, dissolved in 0.5 ml of 1N HCl, and counted after the addition of 5 ml of PCS scintillation cocktail (Amersham). For measurement of DOPAC, 0.1 ml of 5N HCl was added to the reaction mixture from which the methylated catecholamines and DOPEG had been previously extracted. Also added as a cold carrier was 100  $\mu$ g of homovanillic acid (HVA) in 0.1 ml of 0.1M acetic acid, and the radiomethylated DOPAC was extracted into 1 ml of H<sub>2</sub>O-saturated ethyl acetate. The samples were back-extracted into 0.2 ml of 1 mM phosphate buffer (pH 6) and, after the addition of 0.2 ml of 100% ethanol, were evaporated under N<sub>2</sub> at 45° C. The samples were developed on unimpregnated TLC plates for 30 minutes with toluene/acetic acid/ethyl acetate (5:4:1). The spots were visualized, eluted, and counted in the same way as for the catecholamines and DOPEG. The samples were compared to catecholamine and metabolite standards (Sigma) in charcoal-absorbed amniotic fluid. The assay was linear for all five compounds to at least 1 ng/ml (r = 0.99). Assay sensitivity for DA, NE, and E was 2 to 10 pg/tube, and for DOPEG and DOPAC it was 20 to 120 pg/tube. All determinations were made in duplicate. All samples were run in the same assay in order to eliminate interassay variation. The intra-assay coefficient of variation was 4.5% to 12%. Statistical analysis was by analysis of variance and linear regression.

## Results

As shown in Table I, there was an increase in the mean concentrations of E, NE, DA, DOPAC, and

DOPEG in amniotic fluid from the second to the third trimesters of pregnancy. The wide range of values within each trimester did result in considerable overlap between trimesters. When linear regression was used to analyze the relationship between gestational age and the concentrations of catecholamines and metabolites in amniotic fluid, significant positive correlations were noticed between gestational age and the levels of E ( $r = 0.31$ ,  $p < 0.05$ ), NE ( $r = 0.50$ ,  $p < 0.001$ ), DA ( $r = 0.26$ ,  $p < 0.05$ ), DOPEG ( $r = 0.42$ ,  $p < 0.001$ ), and DOPAC ( $r = 0.41$ ,  $p < 0.001$ ). Significant correlations were found between amniotic fluid concentrations of DA and DOPAC ( $r = 0.42$ ,  $p < 0.001$ ) as well as NE and DOPEG ( $r = 0.43$ ,  $p < 0.001$ ) when measured in individual samples.

There were no significant correlations between concentrations of catecholamines or metabolites in amniotic fluid and maternal age, parity, fetal sex, time of amniocentesis, intake of food, number of attempts at amniocentesis prior to obtaining fluid, amniocentesis through the placenta, or contamination of the amniotic fluid with blood.

#### Comment

The present study demonstrates that the concentrations of catecholamines and their metabolic products, DOPAC and DOPEG, are increased in amniotic fluid from mid to late gestation. Several lines of evidence indicate that the catecholamines in amniotic fluid are almost exclusively of fetal origin. With the use of radioactive tracers, both deaminating and O-methylating enzymes have been shown to be abundantly present in the placenta.<sup>2, 3</sup> Consequently, maternal catecholamines are subject to rapid degradation within the placenta before reaching the fetal circulation and amniotic fluid. The concentrations of catecholamines in the umbilical artery are appreciably higher than those in the umbilical vein,<sup>1</sup> which further suggests that catecholamines are synthesized in the fetal compartment and metabolized in the placenta. Transfer of catecholamines to the fetus has been demonstrated in studies in which pharmacologic rather than physiologic amounts of catecholamines were perfused into the maternal surface of the placenta.<sup>16</sup> Although it could be argued that the concentrations of catecholamine metabolites measured represent placental degradation of maternal catecholamines, the finding of a significant correlation between concentrations of DA and DOPAC as well as NE and DOPEG in amniotic fluid would suggest that, under the physiologic conditions represented in this study, these metabolites are also principally derived from the fetus. Thus, the finding of higher levels of catecholamines in the amniotic fluid of late than of mid pregnancy reflects, in all probability, progressive

maturation of the fetal adrenergic system. The concomitant increase in the DA metabolite, DOPAC, and the NE metabolite, DOPEG, would suggest a parallel maturational event in the adrenergic system of the brain and periphery, since the brain appears to be the major contributor to these metabolites.<sup>4-6</sup>

The levels of E<sup>3, 7-9</sup> and DA<sup>10</sup> found in amniotic fluid in the present study correspond well with those previously determined both by chromatography and radioenzymatic assay. However, the concentrations of NE found in the present study are lower than those previously reported,<sup>3, 7-9</sup> except for the single report of Ben-Jonathan and associates.<sup>10</sup> The use of either borate-impregnated TLC plates, as in our study, or two-dimension chromatography, as in the study of Ben-Jonathan and associates,<sup>10</sup> allows the separation of NE from DOPEG. When TLC plates without borate were used, spots representing these compounds were noticed to run together and be counted as a single substance. The combined concentrations of NE and DOPEG in the present study do correspond well with those previously reported for NE alone.

Quantitations in amniotic fluid of DOPEG and DOPAC reported in this study have not been examined previously. Several studies on catecholamine metabolites other than DOPEG and DOPAC support the present finding of adrenergic maturation through pregnancy. Artal and associates<sup>11</sup> measured the concentration of free metanephrine in amniotic fluid, and were unable to detect it prior to 30 weeks' gestation, although appreciable levels were found later in pregnancy. Muskiet and associates<sup>12</sup> found increased concentrations of 3-methoxy-4-hydroxyphenylmandelic acid, HVA, and MOPEG in amniotic fluid throughout gestation. Zambotti and associates<sup>13</sup> found increasing concentrations of MOPEG but decreasing concentrations of p-hydroxyphenylacetic acid throughout pregnancy.

It should be noted that the concentrations of catecholamines reported probably represent free rather than sulfate or glucuronide conjugates, since there is no extraction step prior to radiomethylation in the assay used. Studies carried out by means of acid hydrolysis, or other methods of extraction, prior to measurement have shown higher concentrations of total free E,<sup>7</sup> NE,<sup>7</sup> DA,<sup>10</sup> and MOPEG.<sup>12</sup>

The relevance of peripheral and central fetal adrenergic maturation suggested in this study to the neuroendocrine function of other organ systems and to the initiation of parturition remains to be explored. The findings in this study provide a base for further evaluation of the effect of acute and chronic neurogenic stimuli on the levels of catecholamines in amniotic fluid, particularly their immediate metabolites.



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# Prolactin-stimulated ovarian androgen metabolism

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Activities of  $5\alpha$ ,  $3\alpha$ , and  $3\beta$  reductases were studied with the use of microsomal fractions isolated from the ovaries of immature female rats after treatment with pregnant mares' serum (PMS) followed by human chorionic gonadotropin (hCG). The effect of exogenous prolactin treatment was similarly assessed by comparing enzymic activity in prolactin-treated rat ovaries to that in control rat ovaries, after pretreatment with PMS. With the use of  $^3\text{H}$ -testosterone as substrate, reductase activities were determined by measuring the production of dihydrotestosterone and the  $3\alpha$ - and  $3\beta$ -epimers of androstenediol. The rate of formation of tritiated androstenediol from radiolabeled testosterone was ten times higher than that of tritiated dihydrotestosterone during the follicular phase. After ovulation, the metabolism of testosterone to both androstenediol and dihydrotestosterone decreased markedly. Negligible amounts of estrogen and androsterone were formed, and most of the androstenediol was found to be  $5\alpha$ -androstane- $3\beta$ , $17\beta$ -diol. The Michaelis-Menten constant ( $K_m$ ) for  $5\alpha$ -reductase was  $1.3 \times 10^{-5}\text{M}$ . During the follicular phase, the sum of  $^3\text{H}$ -dihydrotestosterone and  $^3\text{H}$ -androstenediol produced from radiolabeled testosterone was found to be 30% greater in prolactin-treated animals than in control animals. Implications of increased ovarian androgen metabolism as a consequence of hyperprolactinemia are discussed. (AM. J. OBSTET. GYNECOL. 139:487, 1981.)

PREVIOUS STUDIES on androgen metabolism in the rat have yielded conflicting evidence with regard to the possible role of prolactin in the regulation of  $5\alpha$ -reductase activity. After treatment with prolactin,  $5\alpha$ -reductase activity was increased in liver extracts<sup>1, 2</sup> but decreased in adrenal extracts from both male and female rats.<sup>3</sup> It has also been reported that granulosa cells from rat ovaries possess specific prolactin binding sites which are related to increasing progesterone synthesis.<sup>4</sup> However, the relationship between prolactin and the activity of ovarian  $5\alpha$ -reductase in the rat has not been investigated. This study was undertaken to determine the level of androgen metabolites produced by  $5\alpha$ -,  $3\alpha$ -, and  $3\beta$ -reductase in the immature rat ovary and how

they are influenced by the stage of ovarian development and by hyperprolactinemia.

## Material and methods

Intact immature Sprague-Dawley female rats were injected with 50 IU of pregnant mares' serum (PMS; Gestyl, Organon) on the twenty-fifth day of life. Human chorionic gonadotropin (hCG; Ayerst Laboratories), 25 IU, was injected 65 hours after PMS. The animals were killed by decapitation, and the ovaries were dissected free of all areolar tissue and stored in Tris buffer, 0.01M, pH 7.4, at  $-60^\circ\text{C}$  until assay. Hyperprolactinemia was induced in rats with ovine prolactin (National Institutes of Health P-S-11) dissolved in physiologic saline solution at a concentration of 50 IU/ml. Both prolactin-treated and control rats were initially injected with 50 IU PMS at 9 AM on the twenty-fifth day of life and were killed prior to injection of human chorionic gonadotropin (hCG). Prolactin-treated rats were injected subcutaneously with 10 IU of prolactin at 9 AM and 4 PM on the twenty-fifth and twenty-sixth days of life. On the twenty-seventh day, rats were injected at 9 AM and killed at 4 PM.

In all experiments, ovarian microsomal fractions were prepared and reductase activity was determined

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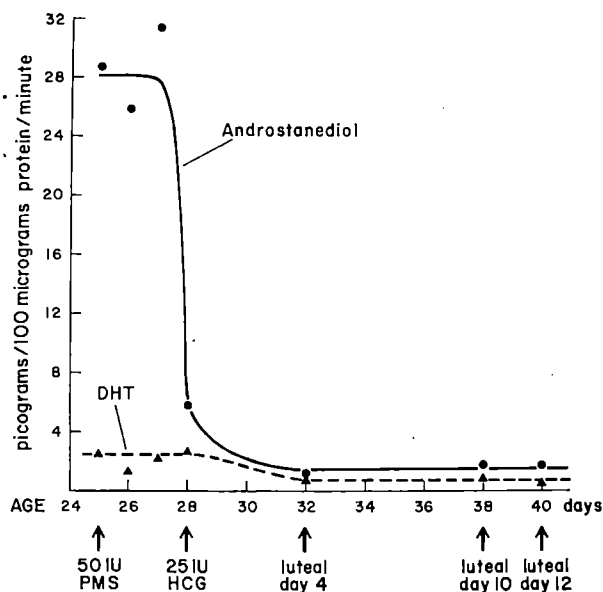


Fig. 1. Initial linear production rates of dihydrotestosterone (---▲---▲---) and of the 3 $\alpha$ - and 3 $\beta$ -epimers of androstenediol (●—●—●—) by ovarian microsomal fractions isolated from 25- to 40-day-old female rats. Initial points represent baseline values from intact 25-day-old animals; subsequent hormonal stimulation is indicated along the abscissa.

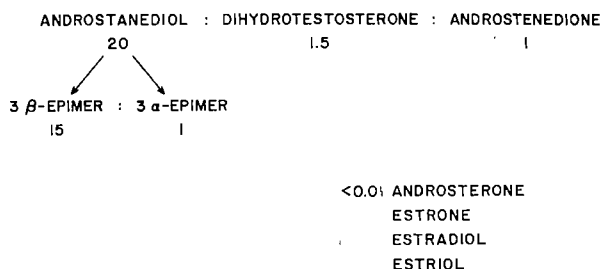


Fig. 2. Molar ratios of androgens produced by ovarian microsomal fractions isolated from rats stimulated with PMS for 48 hours, and molar ratios of estrogens produced by microsomal fractions after 24 hours of PMS stimulation. In all cases, *in vitro* initial reaction velocities were measured.

with the use of a buffered nicotinamide adenine dinucleotide phosphate (NADPH) generating system.<sup>5</sup> Approximately 0.1 to 0.3 gm of ovarian tissue was used in each experiment, so that the final protein concentration was 100 to 150  $\mu$ g protein per reaction mixture.

Michaelis-Menten kinetics were determined with the use of <sup>3</sup>H-testosterone (New England Nuclear lots 697-094 and 1004-021) as substrate over a concentration range of  $10^{-6}$  to  $10^{-5}$ M. Experiments comparing microsomal activity from prolactin-treated animals with that from control animals were carried out under the same reaction conditions<sup>5</sup> at a final testosterone

(Sigma T-1500) concentration of  $7 \times 10^{-6}$ M containing approximately 200,000 cpm per reaction flask of <sup>3</sup>H-testosterone (Amersham Batch No. 30). Initial rates were measured over the first 10 to 15 minutes of the reaction, so that androgen production versus time was linear in all cases. In all experiments, 70% to 90% of the initial tritium counts were recovered in the steroid products.

Products of testosterone reduction were chromatographed with authentic standards on silica gel plates (Analabs Anasil G) in methylene chloride/diethyl ether (75:30) and identified by reacting standards with sulfuric acid followed by heating. Testosterone was completely separated from other androgen metabolites, and the following R<sub>f</sub> values for all steroids isolated were obtained: 5 $\alpha$ -androstane-3 $\alpha$ ,17 $\beta$ -diol, 0.26; 5 $\alpha$ -androstane-3 $\beta$ ,17 $\beta$ -diol, 0.26; testosterone, 0.35; dihydrotestosterone, 0.45; androstenedione, 0.54. Steroids were removed from thin-layer plates by eluting once with methanol and three times with methylene chloride. Samples were then dried and counted in fluor (New England Nuclear Formula-963) with an Isocap 300 scintillation counter.

Separation of dihydrotestosterone and estradiol was effected by developing Anasil G plates twice with the use of a benzene/methanol (85:15) solvent system. Acetylation of androgens was required to separate dihydrotestosterone from androsterone and 5 $\alpha$ -androstane-3 $\alpha$ ,17 $\beta$ -diol from 5 $\alpha$ -androstane-3 $\beta$ ,17 $\beta$ -diol. 5 $\alpha$ -reduced androgens were eluted from Anasil G plates as described after chromatography in methylene chloride/diethyl ether (75:30) and acetylated in a mixture of pyridine, 0.15 cc (Baker Instra Analyzed) and acetic anhydride, 0.15 cc (Baker Instra Analyzed) at room temperature overnight. Reactions were quenched with 1 cc of methanol, solvents were evaporated, and the acetylated androgens were separated on alumina plates (Anasil AG) with the use of benzene/diethyl ether (80:20) as the solvent system.

Protein content of microsomal fractions was determined by the Bradford method.<sup>6</sup>

Except as otherwise noted, reagents were analytical grade.

## Results

**Metabolism of <sup>3</sup>H-testosterone by hyperstimulated rat ovaries.** Production of dihydrotestosterone and a combination of 5 $\alpha$ -androstane-3 $\alpha$ ,17 $\beta$ -diol and 5 $\alpha$ -androstane-3 $\beta$ ,17 $\beta$ -diol by ovarian microsomal fractions with the use of tritiated testosterone as substrate was measured prior to hormonal stimulation and during various stages of follicular and luteal development. As shown in Fig. 1, in unstimulated rat ovaries and

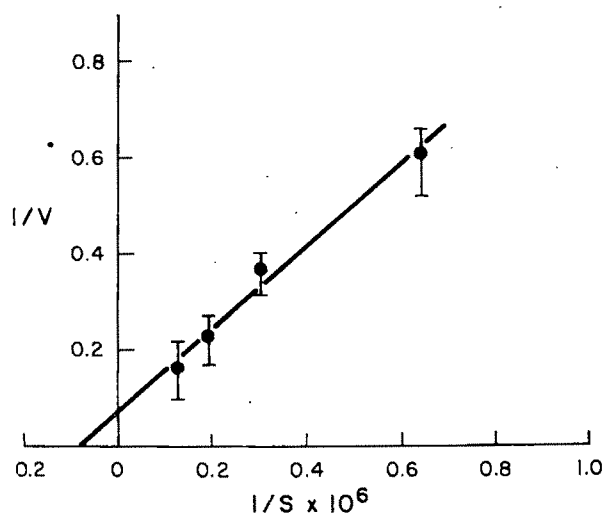


Fig. 3. Lineweaver-Burke plot of 5 $\alpha$ -reductase activity using  $^3\text{H}$ -testosterone as substrate and measuring dihydrotestosterone production. Each point represents an average of the three rates determined prior to and after both 24 and 48 hours of PMS stimulation.  $K_m = 1.3 \times 10^{-6}\text{M}$ .

in follicular phase ovaries, the conversion of  $^3\text{H}$ -testosterone into the radiolabeled 3 $\alpha$ - and 3 $\beta$ -epimers of androstenediol (28 pg/100  $\mu\text{g}$  protein/minute) was approximately ten times that of dihydrotestosterone (2.5 pg/100  $\mu\text{g}$  protein/minute). After induction of ovulation with hCG and throughout the luteal phase, the production of androstenediol decreased to about 2 pg/100  $\mu\text{g}$  protein/minute, and that of dihydrotestosterone to about 0.7 pg/100  $\mu\text{g}$  protein/minute.

Because testosterone can be aromatized to estrogen as well as metabolized to androgen by the reductase enzymes, an attempt was made to determine the production rate of estrogens in this system. Ovarian microsomal fractions from 26-day-old rats were prepared after 24 hours of PMS stimulation and incubated with  $^3\text{H}$ -testosterone. Five micrograms each of carrier estriol and estrone was separated from androgens by thin-layer chromatography; estradiol and dihydrotestosterone cochromatographed in this system were, therefore, eluted and rechromatographed twice on silica gel G plates in benzene/methanol (85:15) to achieve adequate separation. Negligible quantities of all three estrogens were produced compared to the production of dihydrotestosterone under these conditions.

Ovarian microsomal fractions obtained from 27-day-old rats 48 hours after PMS treatment were used to determine the differential production of the 3 $\alpha$ - and 3 $\beta$ -epimers of androstenediol and to quantitate the production of androsterone. Androgens were initially separated on silica gel G plates with the use of a methylene chloride/diethyl ether (75:30) system. Samples

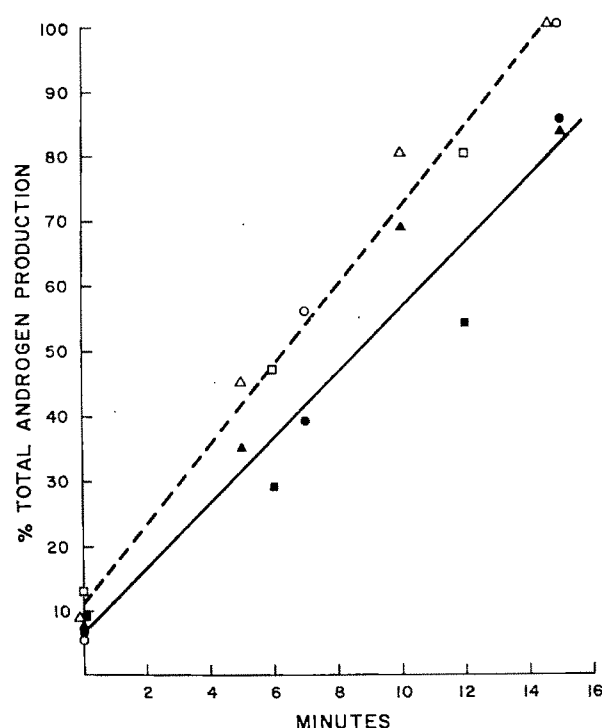


Fig. 4. Percentage of total androgen production versus time for ovarian microsomal fractions from prolactin-treated (----) and control (—) rats.  $\bullet$ ,  $\circ$  and  $\blacksquare$ ,  $\square$  Represent data from animals stimulated for 24 hours with PMS and,  $\blacktriangle$ ,  $\triangle$  represents data from animals stimulated for 48 hours with PMS. All points refer to the amount of  $^3\text{H}$ -testosterone converted to androgen metabolites.  $P < 0.01$ .

were eluted and subsequently acetylated in order to separate androsterone from dihydrotestosterone and to resolve the androstenediols by chromatography on alumina plates in a benzene/diethyl ether (80:20) solvent system. Again, negligible amounts of androsterone, less than 1% of the amount of dihydrotestosterone, were produced. The preponderance of androstenediol was found to be in the 3 $\beta$  form, with a 15:1 molar ratio of 5 $\alpha$ -androsterone-3 $\beta$ ,17 $\beta$ -diol to 5 $\alpha$ -androsterone-3 $\alpha$ ,17 $\beta$ -diol. Fig. 2 summarizes these results.

**Characterization of ovarian 5 $\alpha$ -reductase.** With the use of  $^3\text{H}$ -testosterone as substrate, 5 $\alpha$ -reductase activity in ovarian microsomal fractions was determined by measuring the production of dihydrotestosterone. Michaelis-Menten kinetics were determined over a substrate concentration of  $1.6 \times 10^{-6}\text{M}$  to  $7.7 \times 10^{-6}\text{M}$  testosterone with microsomal fractions obtained from ovaries known to contain high levels of reductase activity.

The production of dihydrotestosterone was measured in microsomal fractions from ovaries of 25-day-old rats prior to injection with PMS, 24 hours after



PMS treatment, and 48 hours after PMS stimulation but prior to injection of hCG. As expected, the kinetics of dihydrotestosterone production were the same regardless of the follicular stage of the ovary. In Fig. 3, a Lineweaver-Burke plot is shown where each point represents an average of the rates determined prior to and after both 24 and 48 hours of PMS stimulation. The  $K_m$  for  $5\alpha$ -reductase was determined to be  $1.3 \times 10^{-5}M$ .

**Androgen production in prolactin-treated rat ovaries.** Rats were treated with PMS on day 25 of life and injected with prolactin as previously described. Ovarian microsomal fractions were isolated from control and prolactin-treated animals 24 and 48 hours after injection with PMS, and incubated with  $^3H$ -testosterone. Since no consistent difference in the relative proportions of androgen metabolites was observed with prolactin-treated animals, results were expressed as total androgen production. Total  $C_{19}$ -reduced androgen production representing the sum of dihydrotestosterone,  $5\alpha$ -androstane- $3\alpha,17\beta$ -diol, and  $5\alpha$ -androstane- $3\beta,17\beta$ -diol production was determined and expressed as picograms of androgen produced per 100  $\mu g$  of protein. Initial reaction rates are shown in Fig. 4 as the percentage of total androgen production over time where all points refer to the amount of  $^3H$ -testosterone converted into androgen metabolites. The dotted line represents androgen production in prolactin-treated animals, and the solid line, that in control animals. Each line is a composite of three separate experiments. It is apparent that  $C_{19}$ -reduced androgen metabolism in prolactin-treated animals was increased by 30% over that in control animals ( $p > 0.01$ ).

#### Comment

Previous studies have demonstrated that large amounts of  $C_{19}$ -reduced steroids are formed by the immature rat ovary,<sup>7, 8</sup> whereas the adult rat ovary produces much lower amounts of these  $5\alpha$ -reduced androgens.<sup>9</sup>  $5\alpha$ -androstane- $3\beta,17\beta$ -diol has also been reported to occur in the peripheral blood of the immature female rat and has been implicated in the onset of puberty.<sup>10</sup> In the present studies, ovarian microsomes from the immature rat, and from animals stimulated with PMS to induce follicular development, metabolized testosterone principally to the  $3\beta$ -epimer of androstenediol. After ovulation, a remarkable decrease of some fifteen-fold in the level of androstenediol was observed. Although others have found  $5\alpha$ -androstane- $3\alpha,17\beta$ -diol,  $5\alpha$ -androstane- $3\beta,17\beta$ -diol, and androstosterone to be the major androgens produced when whole ovarian homogenates were incubated with testosterone,<sup>11</sup> we found very little androstosterone pro-

duced but significant formation of dihydrotestosterone in incubations of testosterone with ovarian microsomes. Levels of dihydrotestosterone produced prior to hormonal stimulation and during ovarian follicular growth were also decreased by about tenfold after ovulation, in parallel with androstenediol. It is probable that  $5\alpha$ -reduced androgens in peripheral blood are of ovarian origin. Since evidence exists that  $5\alpha$ -reductase is under the control of the pituitary gonadotropin luteinizing hormone (LH),<sup>11</sup> it would be interesting to determine whether the enzyme is present in the theca or granulosa compartment or both.

In the present studies,  $5\alpha$ -reductase has a  $K_m$  of  $1.3 \times 10^{-5}M$ , with testosterone as substrate at pH 7.4, which is similar in magnitude to the  $K_m$  of  $8 \times 10^{-5}M$  determined for the enzyme isolated from cultured genital skin fibroblasts at pH 5.5.<sup>12</sup>

Although specific prolactin receptors have been demonstrated in ovarian tissue of many species, including the human,<sup>5, 13, 14</sup> very little is known about the effect of prolactin on ovarian steroid synthesis and metabolism. It has been shown that high levels of prolactin in follicular fluid may depress the synthesis of progesterone by granulosa cells,<sup>15</sup> but no information on the effect of elevated levels of prolactin on either ovarian estrogen or testosterone production and metabolism has been reported. Sulpuride, a drug which produces hyperprolactinemia, increased testicular responsiveness to infusion of chorionic gonadotropin with increased testosterone secretion, but dihydrotestosterone showed little change.<sup>16</sup> Prolactin has been reported to reduce  $5\alpha$ -reductase activity in rat adrenal glands by 20% to 30%.<sup>3</sup> On the other hand, prolactin increased  $5\alpha$ -reductase activity in liver obtained from hypophysectomized rats.<sup>1, 2</sup> Data presented here indicate that prolactin increased  $5\alpha$ -reductase activity in rat ovaries by 30% over that in control animals. Increased androgen metabolism in the presence of hyperprolactinemia may result in follicular atresia either by a direct inhibitory action on estrogen synthesis or by the inability of  $5\alpha$ -reduced androgens to serve as a substrate for estrogen biosynthesis. Androgen receptors have been described in the developing follicle,<sup>17</sup> and androgens may be involved in the luteinization of granulosa cells.<sup>18</sup> Premature luteinization of granulosa cells may lead to anovulation via inhibition of ovulation. Thus, stimulation of  $5\alpha$ -reductase activity with a concomitant increase in  $C_{19}$ -reduced androgen production and reduced estrogen production may result in follicular atresia. This may be one mechanism by which prolactin disrupts normal cyclic ovarian function in the clinical syndrome of hyperprolactinemia, galactorrhea, and amenorrhea.

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# Elevated free cortisol index in pregnancy: Possible regulatory mechanisms

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Biologically active plasma free cortisol increases markedly in pregnancy. In this investigation the free cortisol index (FFI) in the plasma of pregnant and nonpregnant women was measured by a charcoal adsorption technique. The circadian FFI patterns were virtually identical in the two groups, but in gravid women there was a substantial and sustained elevation of the FFI. Sequential studies during gestation and post partum revealed increasing responsiveness of the maternal adrenal glands to adrenocorticotrophic hormone (ACTH) and decreasing suppressibility of the FFI by dexamethasone as pregnancy advanced. Persistence of normal circadian rhythmicity in spite of a continuously elevated FFI and resistance to dexamethasone suppression suggest control of cortisol secretion by normal regulatory mechanisms in pregnancy with resetting of the maternal feedback mechanisms to higher levels. This resetting and the lack of manifestations of cortisol excess in pregnancy might result from tissue refractoriness to cortisol. Elevated free cortisol would be needed to maintain homeostasis. The necessary increase in the production of cortisol could be facilitated by an enhanced responsiveness of the maternal adrenal glands to ACTH. (AM. J. OBSTET. GYNECOL. 139:492, 1981.)

CONCENTRATIONS of plasma cortisol and corticosteroid-binding globulin (CBG) are substantially elevated in pregnancy.<sup>1</sup> Only the unbound cortisol fraction is biologically active.<sup>2</sup> It was assumed in the past that increased plasma cortisol in pregnant women was just sufficient to maintain a normal level of free cortisol,<sup>3</sup> but more recent investigations have indicated that not only total plasma cortisol but also free cortisol is augmented in gravid women, despite the increase in CBG.<sup>1, 4-6</sup> The present studies were undertaken to follow changes in plasma free cortisol during the course of normal pregnancy and to determine whether these changes are governed by normal regulatory mechanisms. Plasma free cortisol was determined by mea-

surement of the free cortisol index (FFI).<sup>7</sup> Responses of the FFI to adrenocorticotrophic hormone (ACTH) and dexamethasone were studied sequentially during gestation and post partum. The circadian patterns of the FFI were compared in pregnant and nonpregnant women.

## Methods

**Subjects and experimental protocol.** Subjects in the study of circadian patterns of the FFI were seven primigravid women in the third trimester of pregnancy and three normal women who were 3 to 12 months post partum, two of whom had been studied previously during gestation. The volunteers were admitted to the Clinical Research Center at least 48 hours prior to the beginning of this investigation and were given regular hospital diets and a prenatal vitamin-mineral supplement. During a 24-hour study period, samples of venous blood of 2 ml or less were withdrawn through an indwelling Teflon catheter at 20-minute intervals, beginning at 0800 hours. After the 24-hour study, an 8-hour infusion of aqueous ACTH, 5 units per hour in 5% dextrose, was given and samples of blood were drawn at frequent intervals before and during the period of infusion. The method for continuous sampling of blood has been described by others.<sup>8</sup> During the

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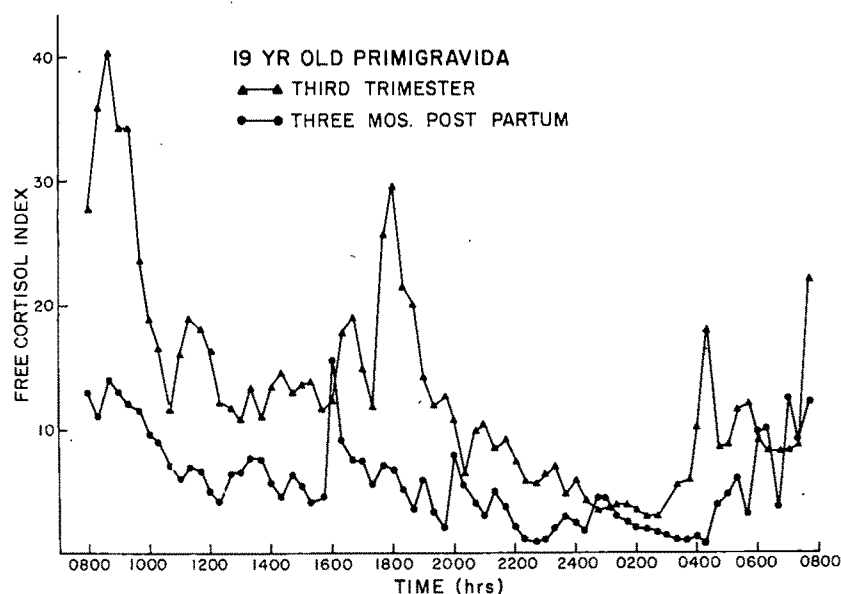


Fig. 1. Patterns of the free cortisol indexes measured at 20 minute intervals during a 24-hour period in the same subject in the third trimester of pregnancy and 3 months post partum.

study, subjects remained supine in bed, except when they ate or used the bathroom.

Another group of seven healthy, ambulatory pregnant women participated in sequential studies of the FFI, before and after short-term stimulation and suppression of cortisol secretion by ACTH and dexamethasone. Single samples of venous blood were obtained at 0800 and 0900 hours, immediately before and 1 hour after intramuscular injection of 0.25 mg of ACTH (Cosyntropin, Organon Inc., West Orange, New Jersey) during each trimester and also post partum. After ingestion of 1 mg of dexamethasone at 2300 hours, another sample of blood was drawn at 0800 hours on the next day.

The suppressibility of cortisol secretion by long-term treatment with dexamethasone was studied in seven third-trimester gravidas. Samples of venous blood for measurement of the FFI were drawn at 0800 hours at the beginning and at the conclusion of a 7-day period of treatment, during which 0.5 mg of dexamethasone was administered every 6 hours.

All gravid women who participated in this study had normal deliveries; none nursed their infants post partum or ingested oral contraceptive agents. The total volume of blood removed during the diurnal studies was 160 ml or less. Plasma was separated by centrifugation within 1 hour after the blood had been drawn. Plasma was stored at  $-20^{\circ}\text{C}$  until assayed.

The study protocol was approved by the Human Research Committee, and informed consent was secured

from volunteers who agreed to participate after being provided with a full explanation of the purpose and the extent of this study.

**Steroid assays.** Plasma cortisol was measured by radioimmunoassay as described.<sup>9</sup> The FFI was measured by a previously published charcoal adsorption method with the use of  $^3\text{H}$ -progesterone as labeled steroid.<sup>7</sup> Separation of protein-bound from free steroid was accomplished by incubation with charcoal-dextran at  $4^{\circ}\text{C}$  and subsequent centrifugation. Percentage binding of labeled steroid was determined by measuring radioactivity in the supernatant. The FFI was derived as follows: (1) Percent free steroid =  $100 - \text{percent binding of } ^3\text{H}\text{-progesterone}$ ; (2)  $\text{FFI} = \text{total plasma cortisol} \times \text{percent free steroid} \div 100$ . Measurements of the FFI by this method were validated by a mathematical approach. By means of a computer analysis described by Feldman and associates,<sup>10</sup> the free cortisol concentrations were calculated in 37 samples of plasma obtained before and after short-term treatment with ACTH or dexamethasone, from third-trimester pregnant and nonpregnant women. In these calculations, affinity constants for binding of competing steroids to various plasma proteins were used in conjunction with representative total steroid and protein concentrations and measured plasma cortisol levels.<sup>9, 10</sup> Calculated data were compared to the FFI measured in the same samples of plasma. A significant positive linear correlation was found between calculated concentrations of plasma free cortisol and measured FFI ( $y = 3.7 x$



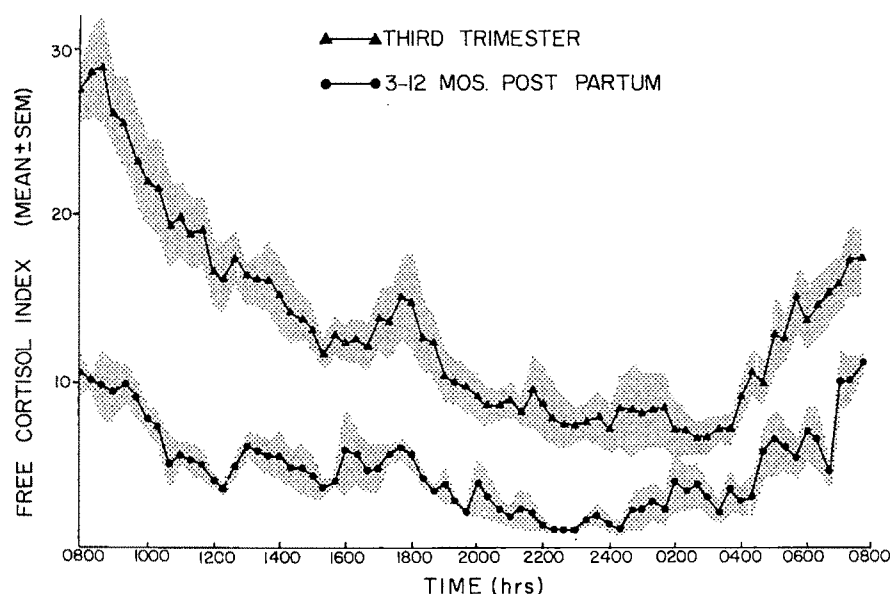


Fig. 2. Patterns of the mean free cortisol indexes measured at 20 minute intervals during a 24-hour period in seven third-trimester gravid women and in three women who were 3 to 12 months post partum.

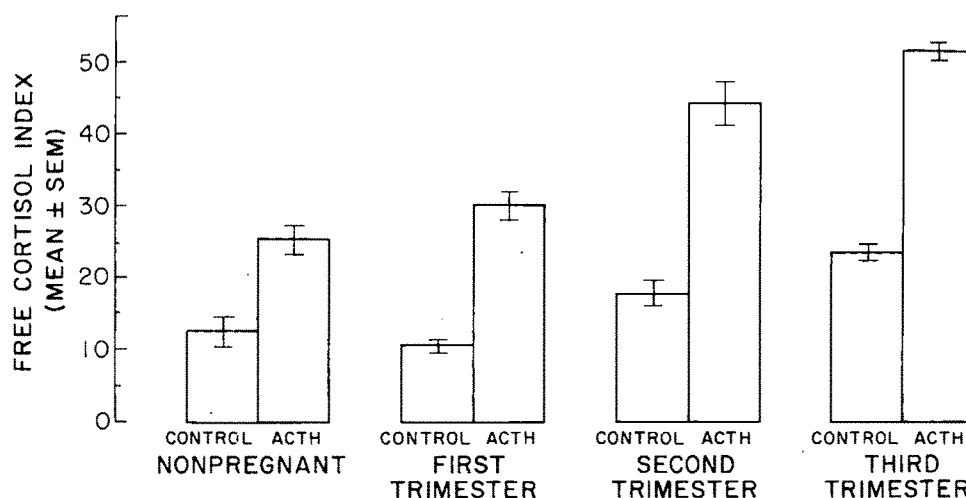


Fig. 3. Mean free cortisol indexes before (CONTROL) and 60 minutes after intramuscular injection of ACTH, measured sequentially in seven women at 0800 and 0900 hours in each trimester of pregnancy and 3 months post partum (NONPREGNANT).

-14.6;  $r = 0.99$ ,  $p < 0.001$ ). This correlation was maintained when the FFI and calculated free cortisol in the plasma of just the dexamethasone-treated subjects were compared ( $r = 0.98$ ,  $p < 0.001$ ). Thus, dexamethasone does not compromise the degree of correlation between the FFI and calculated plasma free cortisol.

Samples from a single patient were processed simultaneously and assayed in duplicate. The significance of differences was determined by the Student's  $t$  test for

paired or unpaired data. Where appropriate, the data were analyzed by an analysis of variance for repeated measurements and missing values. The  $F$  ratio was tested with the most conservative of Box's criteria (degrees of freedom set to 1,6 instead of 3,6); the differences between means were tested by the Newman-Keuls' method adapted for unequal sample sizes.<sup>11</sup> Measurements were reported as the mean  $\pm$  standard error of the mean (SEM).

**Reagents.** 1,2-<sup>3</sup>H-progesterone and 1,2-<sup>3</sup>H-hydro-

cortisone (specific activity, 50 Ci/mmmole) were obtained from New England Nuclear, Boston, Massachusetts. Radioactive agents were at least 97% pure as determined by chromatography. The cortisol antibody used for radioimmunoassay was purchased from Endocrine Science Laboratories, Tarzana, California. Dextran D-4751 was supplied by Sigma Chemical Company, St. Louis, Missouri, and carbon decolorizing alkaline Norit-A was obtained from Fisher Scientific Company, Fair Lawn, New Jersey. All chemicals were analytical grade.

### Results

**Circadian patterns of FFI.** Patterns of FFI for a 24-hour period, measured in the same 19-year-old primigravida in the third trimester of pregnancy and again 3 months post partum, are shown as a representative study in Fig. 1. Similar circadian rhythms of episodic secretory activity were evident in the pregnant and nonpregnant states. In either case the FFI rose to maximal values from 0400 to 1200 hours and declined gradually during the evening toward a nadir between 2300 and 0400 hours. When studied during pregnancy, the afternoon decline in this subject was interrupted by an occurrence of secretory activity from 1600 to 1900 hours. Throughout most of the 24 hour study the FFI was markedly higher in pregnancy than post partum.

Fig. 2 shows the mean patterns of the FFI in seven third-trimester pregnant women and in three subjects who were 3 to 12 months post partum. The plot of mean values obscures the pulsatile nature of cortisol secretion. The circadian patterns of the FFI in gravid and nongravid individuals were similar, but the mean FFI in pregnancy was markedly elevated above nonpregnant values throughout the 24 hour period. At its nighttime nadir the FFI in gravid women approximated peak values measured in nonpregnant individuals, whereas the FFI in the nonpregnant state approached zero.

**Responses of FFI to ACTH.** Mean FFI values measured sequentially in seven normal gravidas during pregnancy and 3 months post partum, before and after intramuscular injection of 0.25 mg of ACTH, are shown in Fig. 3. The mean baseline FFI at 0800 hours was  $12.4 \pm 2.2$  in the nonpregnant state. The FFI apparently did not increase in early pregnancy. Its steepest rise from  $10.6 \pm 1.2$  to  $18.0 \pm 1.9$  occurred between the first and second trimesters ( $p < 0.05$ ). The FFI plateaued in the third trimester at  $23.7 \pm 1.3$ , which was significantly greater than its level in the nonpregnant state and in the first trimester ( $p < 0.01$ ). The responsiveness of the FFI to short-term stimula-

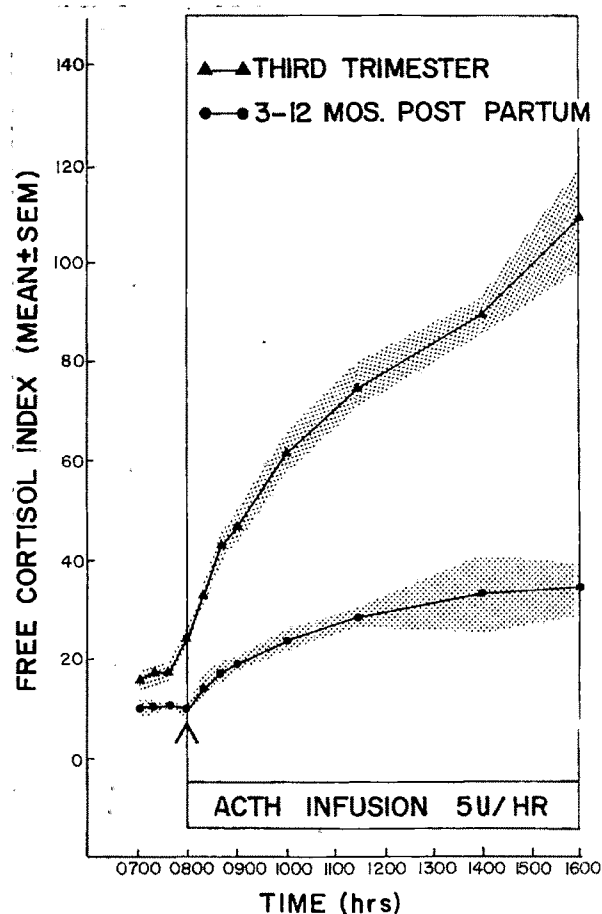


Fig. 4. Effects of an 8 hour infusion of ACTH upon the mean free cortisol indexes in seven third-trimester gravid women and in three women who were 3 to 12 months post partum.

tion with ACTH increased as pregnancy advanced and reached a plateau after the second trimester: the mean ACTH-induced FFI increment was  $13.0 \pm 3.5$  in the nonpregnant state,  $19.1 \pm 2.1$  in the first trimester,  $26.3 \pm 3.2$  in the second trimester, and  $28.1 \pm 1.8$  in the third trimester. The increase in the FFI after ACTH was significantly greater in the pregnant than in the nonpregnant state (first trimester,  $p < 0.05$ ; second and third trimesters,  $p < 0.01$ ). The increment in the second trimester approached a significant difference from measurements in the first trimester ( $p \approx 0.06$ ). The increment in the third trimester was significantly greater than that in the first trimester ( $p < 0.05$ ), but did not differ from values measured in the second trimester.

Fig. 4 shows FFI measurements before and during an 8-hour infusion of ACTH (5 U/hr) in seven third-trimester pregnant women and in three subjects who were 3 to 12 months post partum. In nongravid women the mean FFI increased during infusion of ACTH



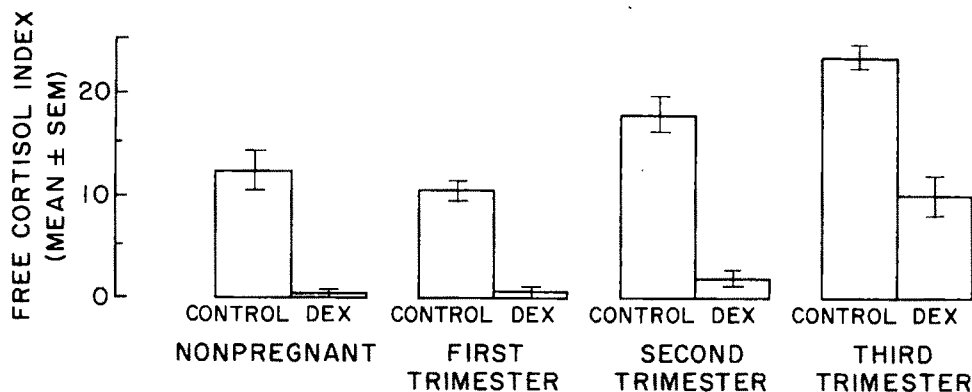


Fig. 5. Mean free cortisol indexes measured sequentially in seven women in each trimester of pregnancy and 3 months post partum (*NONPREGNANT*) at 0800 hours before (*CONTROL*) and on the morning after ingestion of a single nighttime dose of dexamethasone (*DEX*).

from  $10.4 \pm 1.2$  to  $35.2 \pm 5.4$ , whereas in gravid women it rose from  $24.0 \pm 1.6$  to  $109.6 \pm 10.0$ . The difference in the FFI response to ACTH, with larger increments in pregnant than in nonpregnant women, reached significance at 40 minutes into the study ( $p < 0.05$ ) and continued to increase throughout the course of infusion.

**Responses of FFI to dexamethasone.** The suppressive effect of a single nighttime dose of 1.0 mg of dexamethasone on the FFI measured at 0800 hours was studied sequentially in the same seven gravid women during the course of pregnancy and 3 months post partum (Fig. 5). As baseline values of the FFI rose with advancing gestation, dexamethasone suppression became less effective: the mean FFI declined with dexamethasone to  $0.4 \pm 0.1$  in the nonpregnant state, to  $0.7 \pm 0.1$  in the first trimester, to  $2.0 \pm 0.9$  in the second trimester, and to  $10.5 \pm 1.9$  in the third trimester. Analysis of variance of the FFI values measured after treatment with dexamethasone showed that the FFI in the third trimester was significantly greater than all other values ( $p < 0.01$ ), and that levels in the second trimester were significantly greater than in the nonpregnant state and first trimester ( $p < 0.01$ ). Hence, one can conclude that the dexamethasone suppressibility of the FFI decreases progressively as pregnancy advances.

Long-term treatment of seven third-trimester gravid women with 0.5 mg of dexamethasone every 6 hours for 7 days caused a decline in the mean FFI from  $25.2 \pm 2.2$  to  $3.2 \pm 1.2$ . This suppressed value measured in pregnant women after prolonged treatment was significantly higher than the mean FFI determined in seven nongravidas after administration of a single nighttime dose of dexamethasone ( $0.4 \pm 0.1$ ;  $p < 0.05$ ).

### Comment

In this study, unbound metabolically active plasma cortisol was estimated by a simple charcoal adsorption technique and expressed as the FFI.<sup>7</sup> This method had been validated previously by demonstrating that the percentage of free steroid determined by charcoal adsorption shows a direct linear correlation with the percentage of free steroid determined by equilibrium dialysis in the same samples of plasma.<sup>7</sup> The present investigation further validated the charcoal adsorption technique by demonstrating that the FFI is significantly correlated with the calculated free cortisol concentration derived from a computer analysis which took the presence of cross-reacting ligand-binding systems into account. Since the FFI is measured accurately in very small plasma aliquots, it offers a useful means of determining relative differences in the concentration of free cortisol in the limited volume of blood that could be withdrawn safely at each of the numerous samplings required by the study protocol.

In pregnancy, a circadian FFI pattern was apparent that was virtually identical not only to the FFI pattern in the nonpregnant state but also to the pattern of total cortisol in nongravid women described by us and others.<sup>8,9</sup> In both pregnant and nonpregnant women, the highest FFI levels occurred early in the day and resulted from episodic bursts of secretory activity which waned toward evening, when the FFI declined to its nadir. The major difference between the diurnal patterns in the two groups of women was an elevation of the FFI in the pregnant subjects that was sustained throughout the circadian cycle. Others who used equilibrium dialysis and centrifugal ultrafiltration at 37°C or steady-state gel filtration have shown that plasma free cortisol in gravid women is elevated at specific times of the day.<sup>1,4-6</sup> The present study extends these

previous reports by providing evidence that third-trimester pregnant women are exposed to elevated levels of unbound, metabolically active cortisol at all times of the day. Moreover, the similarities in the circadian FFI patterns of pregnant and nonpregnant women suggest that the secretion of cortisol is regulated by normal mechanisms in gestation.

Our previous studies showed that the elevation of free cortisol in gravid women results from prolonged plasma half-life and enhanced production of cortisol.<sup>9</sup> The delayed clearance of cortisol in pregnancy has been explained by increased plasma levels of CBG which would tend to reduce the fraction of circulating free cortisol that is immediately available for metabolism. The mechanisms that lead to an increased production of cortisol in pregnancy are, however, not readily apparent. The present investigation suggests that the adrenal cortex in gravid women is more readily stimulated to secrete cortisol. Increments in the FFI after injection of a single dose of short-acting ACTH more than doubled as pregnancy advanced. This increase in the responsiveness of the adrenal glands to stimulation during gestation was even more obvious when ACTH was administered by long-term infusion. Johnstone and Campbell<sup>12</sup> reported comparable findings after maximal stimulation of the adrenal glands with a single dose of long-acting ACTH in third-trimester gravid women. Since ACTH is thought to be elevated during pregnancy, our findings of enhanced responsiveness to ACTH are consistent with the well-known observation that the corticosteroid response of the adrenal glands increases with prior exposure of them to ACTH.<sup>13, 14</sup>

The gradual decrease in suppressibility of the FFI by dexamethasone as pregnancy advanced might be explained by several mechanisms. A fraction of the maternal ACTH could be produced by an extraneous source that does not respond to suppressive maneuvers. This hypothesis is supported by the reports of Genazzani<sup>13</sup> and Rees<sup>14</sup> and their coworkers that a component of maternal ACTH is not of pituitary origin but, rather, is produced by the placenta. The release of placental ACTH would be autonomous and, therefore, not subject to feedback control. Moreover, the fetus might contribute substantially to the maternal cortisol pool, as suggested by reports of lowered cortisol excretion values associated with anencephalic pregnancies.<sup>15</sup> If the fetal source of cortisol responded less readily than the maternal adrenal glands to dexamethasone suppression, this could contribute to the loss of suppressibility of the free cortisol fraction in pregnancy. Earlier investigators did notice suppression of the fetoplacental production of estrogens and their

adrenocortical precursors after the administration of corticoids to pregnant women; however, the doses given were considerably higher than those employed in this study.<sup>16-18</sup> It is quite unlikely that the delayed plasma clearance of cortisol would account for the impaired suppressibility of the FFI, since even long-term treatment of third-trimester gravidas failed to lower the FFI appropriately.

Neither increased responsiveness of the adrenal glands to ACTH nor decreased suppressibility of the FFI by glucocorticoids would of themselves result in increased production of cortisol by the maternal adrenal glands unless the hypothalamic-pituitary regulation of cortisol production had been changed. Also, any sustained increase in the FFI by an extraneous source of cortisol or ACTH would be expected to attenuate or even abolish the normal nyctohemeral pattern of cortisol secretion, but, as noted above, patterns in pregnancy were very similar to those in nonpregnancy.

A possible explanation for the persistence of normal circadian rhythmicity in the face of a continuously elevated FFI, and also for the resistance to suppressive doses of dexamethasone in pregnancy, is that the maternal pituitary-hypothalamic feedback mechanisms may be reset at a higher level and, thus, be less sensitive to the suppressive effect of glucocorticoids during gestation. This resetting of the feedback mechanisms of cortisol secretion and, also, the well-known lack of clinical manifestations of cortisol excess in normal pregnancy might result from the development of tissue refractoriness to the effects of cortisol. Thus, elevated levels of free cortisol would be needed to maintain homeostasis, and increased sensitivity of the maternal adrenal glands to ACTH would facilitate the necessary increase in the production of cortisol. Accordingly, it seemed to be reasonable to speculate that refractoriness of target tissues to glucocorticoids might be associated with diminished binding of cortisol to specific intracellular glucocorticoid receptors. In keeping with this hypothesis, preliminary results of recent *in vitro* studies indicate that specific binding of radioactively labeled dexamethasone to intact lymphocytes is significantly reduced in the presence of progesterone, presumably because of competitive binding of progesterone to glucocorticoid receptors.<sup>19</sup> It is assumed that the high levels of circulating gestational progestins would exert a similar inhibitory effect and, thus, account for the increase in plasma free cortisol.

We are indebted to William E. Bridson, M.D., Primate Research Center, and Warren H. Dennis, Ph.D., Department of Oncology, University of Wisconsin, for their helpful advice. We also would like to thank Ms. Terri Kraemer for her invaluable secretarial assistance.



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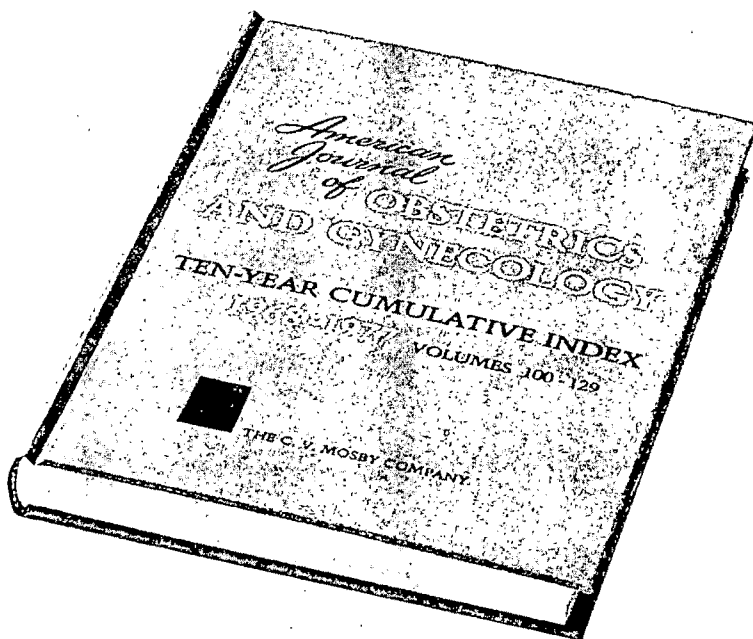
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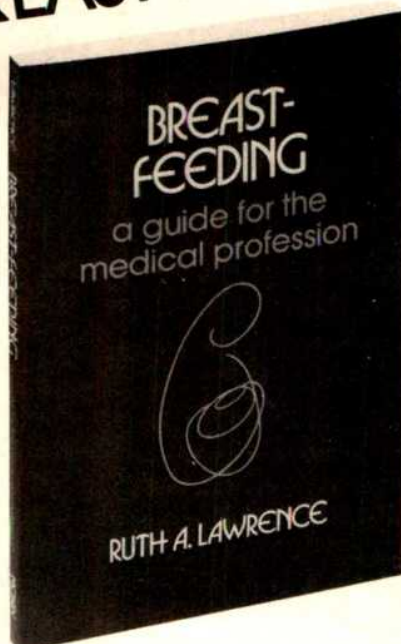
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**Sure as a new day will dawn,  
Peri-Colace<sup>®</sup> works with gentle, proven predictability.**

PERI-COLACE combines the gentle laxative and effective stool softener so often needed when bowel motility is depressed. PERI-COLACE acts gently to induce peristalsis, while the stool-softening agent lets natural intestinal water permeate stools.

PERI-COLACE is predictable—gentle evacuation usually occurs within 8 to 12 hours. It is useful in obstetrical patients, as well as in geriatrics, pre- and postoperative patients, the convalescent, in patients with medication-related suppression of bowel motility and children.

PERI-COLACE—gentle, proven, predictable. In capsule and liquid forms.

**PERI-COLACE**

casanthranol and dioctyl sodium sulfosuccinate  
*Gentle laxative plus stool softener*

**Mead Johnson**

PHARMACEUTICAL DIVISION